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IMPACT OF GLYPHOSATE DRIFT ON NON-TARGET
FIELD MARGIN INVERTEBRATES

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A thesis submitted in partial fulfilment of the requirements of The Open
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ABSTRACT

Grassy arable field margins provide important permanent habitats for arthropods in agro-ecosystems and due to their proximity to high input areas, are exposed to pesticide drift. The aims of this thesis are to determine the likely effects of glyphosate drift in arable field margins by examining patterns of a medium quality spray drift intercepted by plant species in buffer strips and the effects of glyphosate on non-target field margin arthropods. Levels of medium-quality spray drift, analogous to herbicide drift, intercepted by field margin plant species in field boundaries were significantly reduced by inclusions of 2m and 6m wide buffer strips. Levels of spray drift interception varied between plant species and were related to plant height and leaf area. Dose-response testing of glyphosate against field margin plant species was done to establish inherent susceptibilities to the herbicide. Many species appeared to be unaffected by high levels of glyphosate (1800g ha^{-1}), while others had relatively high ED_{50}s that were unlikely to be exceeded by UK recommended rates of glyphosate. It was noted that lack of exposure to interspecific competition may have enhanced the plant species tolerance to high levels of glyphosate. Different rates of glyphosate were screened against the non-target arthropods *Lepthyphantes tenuis* (Araneae) and *Leptopterna dolabrata* (Heteroptera) to assess toxicity. Glyphosate was found to be non-toxic, however, applications of glyphosate to food plants increased mortality in *L. dolabrata*. In a field experiment, glyphosate applications of more than 360g ha^{-1} to a grassy arable field margin reduced Araneae, Heteroptera and Carabidae abundance. Community analyses (DCA) indicated that communities in the field margins exposed to more than 360g ha^{-1} glyphosate were distinct from unsprayed field margins. It is predicted that drift of field applied glyphosate at rates greater than 1440g ha^{-1} would reduce phytophagous Heteroptera and *Gonatium rubens* (Araneae) abundance.

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- Appendix 2 Araneae Species Recorded from the Field Margins at Loddington in this Study**
- Appendix 3 Carabidae Species Recorded from the Field Margins at Loddington in this Study**
- Appendix 4 Heteroptera Species Recorded from the Field Margins at Loddington in this Study**

LIST OF PUBLISHED MATERIAL

Haughton, A.J., Wilcox, A., Chaney, K., Cooper, S.E. & Boatman, N.D. (1998). Spray drift into field margins: The effect of width of buffer strip and plant species on the interception of spray drift. *BCPC - Pests and Diseases* 1, 285 - 290.

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Haughton, A.J., Wilcox, A., Chaney, K., & Boatman, N.D. (1999). The effects of different rates of glyphosate on non-target invertebrates in field margins. *Aspects of Applied Biology* 54, 185-190.

OVERVIEW OF THESIS

Use of the herbicide, glyphosate, in arable crops in the UK rose by more than 300% during the period 1996-1998, and further rises are predicted if permission for growing herbicide resistant crops is granted. The effects of herbicide drift into grassy arable field margins on non-target arthropods has not been investigated. The effects of herbicide drift on plant communities has been shown to reduce abundance and diversity of non-weed species and thus, it is possible that changes in flora may influence arthropod species abundance and community structure.

In order to investigate the effects of herbicide drift on non-target field margin arthropod species and community structure, this thesis comprises 5 experiments designed to study the different aspects of herbicide drift and possible routes of impact on arthropods. Firstly, an experiment was done to quantify the interception of spray drift by different plant species in different widths of buffer strip (Chapter 3). Next, dose response testing of glyphosate against most of the species from the previous experiment was done to determine specific susceptibilities to the herbicide (Chapter 4). Different aspects of leaf characteristics were examined to establish whether they influenced either spray drift interception and/or effect of glyphosate.

To test whether glyphosate affected the quality of food plant for a species of Heteroptera a feeding experiment was conducted (Chapter 5). Also, toxicity testing of glyphosate against a species of Heteroptera and Araneae was done to establish whether glyphosate had insecticidal properties (Chapter 6).

In the final experiment, arthropods were sampled from a grassy arable field margin sprayed with rates of glyphosate and changes in arthropod group and species abundance and community structure (Araneae, Carabidae and Heteroptera) were recorded (Chapter 7).

The implications of the experimental work were discussed in the context of estimating the likely effects of glyphosate drift on non-target arthropods in grassy arable field margins.

1 GENERAL INTRODUCTION

1.1 Field Systems - Historical Context

1.1.1 Fields & Boundaries

Fields and their boundaries have existed ever since civilisation began (Rackham, 1996). Field boundaries form demarcations between fields and generally comprise hedgerow, wall, ditch, grassy strip or bank, either alone or in combination (Bunce *et al.*, 1994; Rackham, 1996).

The oldest known field boundary system in the British Isles is one characterised by a pattern of low stony banks called reaves (Rackham, 1996). The oldest reaves are found in County Mayo, and have been dated to the Neolithic (Caulfield, 1978). In Britain, however, the oldest reaves are found in lowland England dating from the Bronze Age (Rackham, 1996).

Although the earliest fields were characterised by largely inorganic, irregular boundaries, it seems that the hedgerow was beginning to be used as a boundary in Europe before Roman times. The earliest written record of the hedge is by the agricultural writer Columella in the first century BC (Rackham, 1996) and the first records of British hedges are found in the Anglo-Saxon Chronicles (Pollard *et al.*, 1974; Rackham, 1996). Furthermore, traditional hedge-laying skills, indicating active hedgerow management, had already been well established by a tribe encountered by Caesar in Flanders, (Pollard *et al.*, 1974; Rackham, 1996).

The most abundant type of living field boundary in Britain is the hedge (Barr *et al.*, 1993). Current definitions of hedges and hedgerows used by ecologists classify the hedge as the

structure formed by shrubs and trees, whereas the hedgerow is the hedge structure, plus associated tall and short herb layers (Greaves & Marshall, 1987; Marshall, 1988; Barr *et al.*, 1991). The earliest hedges made boundaries of properties and settlements (Rackham, 1996), however, since around Anglo-Saxon times field boundaries have been used for the impoundment and delineation of stock (Pollard *et al.*, 1974; Rackham, 1996).

The abundance of hedgerows increased following the Enclosure Acts of the 18th and 19th Centuries (Rackham, 1996), but the 20th Century post-war years have witnessed an unprecedented increase in hedgerow removal (Pollard *et al.*, 1974). During the period 1978 - 1984, 28 000 km of hedges were lost (Barr *et al.*, 1986), and during the period 1984 - 1990 loss of boundaries containing a hedgerow was in the region of 131 000km (Barr *et al.*, 1993).

1.1.2 Arable Field Margins

Arable field margins are composite boundaries and comprise three elements: the boundary, boundary strip and crop margin and, as such, arable field margins occupy the area between the field boundary and the first tractor tramline (Greaves & Marshall, 1987). The boundary comprises the barrier, which may be a hedge, fence, wall, hedge bank or ditch and associated herbage, while the boundary strip, which is the area between the boundary and the crop, may include a farm track, grassy strip, and/or unsown cultivated sterile strip (Figure 1.1). The crop margin, which is the area between the crop edge and the first tractor tramline, is now commonly referred to as the crop 'headland' (Jones *et al.*, 1991; Boatman, 1994). Where only selective pesticides are applied to the headland area of the crop, these areas are known as 'conservation headlands' (Sotherton, 1991).

1.2 Ecological & Agricultural Importance of Arable Field Margins

Agriculture is the dominant land use in Britain, with 80% of the land area in agricultural use (Anon, 1990) and arable land covering 34% of the area of Great Britain (Barr *et al.*, 1993). With such a high coverage, farmland is, therefore, extremely important in the context of wildlife conservation in Britain. Furthermore, the move away from extensive, low input traditional farming towards an intensive, high input regime has put increased pressures on agro-ecosystem flora and fauna due to pesticide use, habitat fragmentation and species isolation (Webb & Haskins, 1980; International Union for the Conservation of Nature, 1983; Baldock, 1990; Ravenscroft, 1990). Because field margins often represent the only uncropped area on the farm and may comprise, in any combination, three key elements of semi-natural habitat, i.e., woodland (hedge), wetland (ditch) and grassland (verge or bank) (Hooper, 1987) the representative flora and fauna can be diverse. The European Research Network on Field Margin Ecology co-ordinated Europe-wide research into the management and ecological function of field margins and has concluded that field margins play a key role in agroecosystem ecology, but are exposed to harmful farming practices (Marshall & Moonen, 1998). Arable field margins are important refuges and corridors for species migration, colonisation and dispersal (Dennis & Fry, 1992), and are not only important in terms of biodiversity and large-scale ecological processes, but also for farming.

Integrated pest management (IPM) is part of the whole farm approach to ecologically sustainable farming practice, known as Integrated Crop Management. IPM addresses pest control problems within a crop and utilises the disciplines of applied entomology, plant pathology, weed science and nematology to achieve pest management strategies that are practical, economical and protective of public health and the environment (Dent, 1995).

Field margins dominated by perennial plant species are useful in IPM since they can exclude many agricultural annual weeds (Marshall & Moonen, 1997) without increasing weed occurrence in adjacent crops (Smith *et al.*, 1999). Field margins also support (Sotherton, 1983; Dennis & Fry, 1992) and can enhance (Dennis 1991; Dennis & Fry, 1992; Wratten *et al.*, 1998) populations of natural enemies of pests. These natural enemies play a vital role in IPM since they reduce pest numbers (Dennis & Wratten, 1991), for example, Leather (1993) found that natural enemies were the major factor causing aphid egg mortality. Therefore, augmentation of natural enemies is an important concept in IPM since they can reduce the need for insecticides by reducing pest numbers.

Confirming that less intensively farmed land is valuable for wildlife and nature conservation (Halley & Lawton, 1996), cereal field margins are now included as a priority habitat in the UK Biodiversity Action Plan (Anon, 1995a). In order that the conservation and enhancement of biodiversity in field boundaries and margins may be facilitated, farmers are paid for appropriate management of these features within the Countryside Stewardship (MAFF, 1996), some Environmentally Sensitive Areas (MAFF, 1994) and the pilot Arable Stewardship scheme (MAFF, 1998) agri-environment schemes. Here, the importance of arable field margins to flora and arthropod fauna is reviewed.

1.2.1 Flora

Compared with cropped areas, the flora associated with uncropped areas of field margins is both distinct and diverse (Cummins *et al.*, 1992; Marshall & Arnold, 1995; Wilson & Aebischer, 1995; Kiss *et al.*, 1997), while the vegetation communities of the grassy strip element of arable field margins (Cummins *et al.*, 1992) are typical of mesotrophic grassland, especially the MG1 community of the National Vegetation Classification

(Rodwell, 1992). Although these communities are not outstanding in terms of nature conservation value, arable field margins have been identified as the most botanically interesting area on farmland (Rands & Sotherton, 1987; Wilson & Aebischer, 1995), acting as refuges for many arable weeds (Wilson 1991; Wilson & Aebischer 1995; Marshall & Arnold, 1995; Kiss *et al.*, 1997). However during the period 1978 - 1990 there was a loss of botanical diversity, which was attributed to both neglect and intensive management of boundary features (Bunce *et al.*, 1994).

Arable field margin habitats are especially important for arable weeds, however, the removal of field margins has been cited as contributing to the decline of these arable weeds (Wilson, 1994). Since most arable weeds have annual life cycles and depend on regular cultivation, low soil fertility and lack of herbicide use (Wilson, 1991), they tend to be found in the headland areas of the crop (Wilson & Aebischer, 1995). However, many arable weeds have become increasingly rare: in a survey of plants occurring in arable land, it was found that 6 species that had become extinct since 1960 and 23 species had become endangered (Wilson, 1991). Rare species such as Rough poppy (*Papaver hybridum* L.) and Narrow-fruited cornsalad (*Valerianella dentata* (L.) occur more frequently in cropped headlands where agrochemical input is reduced (Wilson, 1991).

1.2.2 Arthropod Fauna

Field margins have been shown to support important populations of diverse non-pest and beneficial arthropods by providing a permanent, stable and complex habitat (Dennis & Fry, 1992) and in some instances, field margins have been found to influence the arthropod composition of adjacent hedges (Maudsley *et al.*, 1997). Field margins and conservation headlands harbour a significantly more dense and diverse arthropod fauna than the crop

itself (Lewis, 1969; Glück & Ingrisch, 1990; Dennis, 1991; Hassall *et al.*, 1992; Kielty *et al.*, 1992; Frank & Nentwig, 1995; Kiss *et al.*, 1997, etc), while the boundary features of arable fields are important for arthropod dispersal and over-wintering.

Although arthropod communities of arable crops are typically those that can disperse rapidly (Morris & Webb, 1987), field margins provide permanent areas from where dispersal may take place. Petit & Burel (1998) found that the hedgerow network provided an important route for dispersal of *Abax parallelepipedus* (Piller & Mitterpacher) (Coleoptera: Carabidae) within a farmed landscape and crop edges have been shown to be bases for dispersal of carabid beetles into adjacent crops (Coombes & Sotherton, 1986; Duelli *et al.*, 1990). Dispersal of arthropods is an important aspect of the dynamics of recovery from farming practices such as harvesting and pesticide application. Thomas *et al.* (1990) observed the recovery of two species of linyphiid spider from a field application of an insecticide, and found that dispersal into the sprayed area occurred from the unsprayed crop and non-crop areas.

Field margins represent important overwintering sites for arthropods. Desender *et al.* (1989) note that grassy margins are especially important to carabid beetles with low dispersive capabilities. Certain features within field margins are more beneficial than others for arthropod overwintering. Tussock-forming grasses, for example, are preferred to non-tussock species by many arthropods (Luff, 1966), and *Tachyporus hypnorum* (F.) (Coleoptera: Staphylinidae) and *Demetrias atricapillus* (L.) (Coleoptera: Carabidae) have higher survival rates in tussock grass species (Dennis *et al.*, 1994). Preferences between tussock grass species have also been observed. For example, the density of lycosid spiders was greater in *Dactylis glomerata* L. and *Deschampsia caespitosa* (L.) grasses than in *Holcus lanatus* L. and *Festuca rubra* L. grasses (Bayram & Luff, 1993).

The increased plant diversity of field margins, compared with the crop, is largely responsible for determining the quality of arthropod populations by providing diverse structural composition and a variety of food sources. For example, increased vegetation structural complexity, which is correlated with an increase in plant diversity (e.g., Brown, 1991), is an important determinant of an enhanced spider community structure (White & Hassall, 1994). Many of the plants found within arable field margins support a diversity of non-pest arthropods. Loss of forage for important farmland pollinators has been cited as a cause for a recent decline in bumble and honey bees (Osborne & Corbet, 1994) since field margins can provide the necessary cover of perennial herbs required by pollinators. Indeed, flower-rich field margins support a diversity of pollinating insects (Lagerlöf *et al.*, 1992), most notably hoverflies (Diptera: Syrphidae) (Cowgill *et al.*, 1993; Sutherland & Poppy, 1997), bumblebees (Hymenoptera: Apidae) (Fussell & Corbet, 1992; Dramstad & Fry, 1995; Saville *et al.*, 1997) and satyrid Lepidoptera (Feber *et al.*, 1995, 1996, 1997).

Field margin habitats also support arthropods that are important prey items for fauna in higher trophic levels. Most notable are soft-bodied insects, such as Symphyta larvae and Hemiptera, which are preyed upon by gamebird chicks (Chiverton, 1999) and other farmland birds (Wilson *et al.*, 1997a). Indeed, the abundance of arthropod prey items in field margin habitats is known to be important for farmland bird conservation (Southwood & Cross, 1969; Rands & Sotherton, 1987; Anon, 1995b; Wilson *et al.*, 1997b).

1.3 Pesticide Use in the Agro-ecosystem

1.3.1 Historical Perspective

Pests and diseases have always been a problem in agriculture. Food losses in Africa caused by cereal rusts and locust plagues more than two thousand years ago still occur in

Africa today (Cremlyn, 1991). In Britain, one of the first organic pesticides was developed in 1850 (Cremlyn, 1991) and hitherto, pesticide research, development and use have continued to increase.

Pesticide use increased dramatically following the end of World War II, initially in response to the drive to make Britain self-sufficient in food supply. Sly (1977), in a review of the use of pesticides, noted that in 1945 there were less than 20 active ingredients used in pesticides and by 1975 this figure had increased to 200. Although many active ingredients have been replaced with more modern, safer chemicals, it is estimated that there are approximately 1,000 pesticide formulations in use throughout the world, and these are used for almost all forms of agricultural commodity (Albert *et al.*, 1992).

1.3.2 Current Pesticide Usage

It is generally accepted that pesticides need to be used in agriculture if food production is to meet human demands. Cereals account for the largest cropped area in Britain (Longley & Sotherton, 1997a) and pesticide use in the cereal ecosystem is still characterised by high levels of prophylactic use (Burn, 1987; Longley & Sotherton, 1997a). Table 1.1 gives the estimated amounts of insecticides, fungicides, herbicides and total pesticides applied to the area of treated arable land in four cropping seasons since 1990. The area to which all groups of pesticide were applied increased from 1994 to 1998, however, the amount has remained relatively static due to the increased practice of reduced application rates and the introduction of new products active at lower rates of application (Thomas *et al.*, 1997).

Table 1.1. Area of arable farm crops (ha x 10⁶) treated with insecticide, fungicide, herbicide+desiccant and total pesticides in 1992, 1994, 1996 and 1998.

	Insecticide	Fungicide	Herbicide+desiccant	All Pesticides
1992	3.6	12.1	10.3	33.9
1994	3.1	10.8	10.9	32.5
1996	4.4	13.5	12.4	38.3
1998	4.3	11.8	14.1	42.9

After Davis *et al.*, 1993a; Garthwaite *et al.*, 1995; Thomas *et al.*, 1997, D. Garthwaite, pers. comm., Pesticide Usage Group, Central Science Laboratories, York, 1999

Pesticides are not only applied to the crop. Use of herbicides in set-aside contributes greatly to the overall area of land treated with pesticides. In 1998, for example, 17% of glyphosate applied to farmland was sprayed on set-aside (D. Garthwaite, pers. comm.). Many farmers perceive field margins as harbours of weeds, pests and diseases (Marshall & Smith, 1987) and this view has led to the deliberate spraying of field edges and boundaries (Marshall & Birnie, 1985; Wilson & Aebischer, 1995). Indeed, a survey of Dutch farming practice revealed that 85% of farmers intensively spray the crop edges with herbicide (de Snoo, 1994).

1.3.3 Herbicides and Non-Target Arable Flora

Since the 1940s, diversity in arable plant communities and several arable weeds have suffered major declines due to changes in, and intensification of farming practice, including increasing herbicide and fertiliser use (e.g., Chiverton & Sotherton, 1991; Wilson, 1991; Smith & Macdonald, 1992; Kleijn, 1996; Kleijn & Snoeijs, 1997). For example, where herbicides had never been applied to an experimental winter wheat crop at Rothamsted Experimental Station, UK, rare arable weeds including Corn Buttercup, *Ranunculus arvensis* L., Spreading Hedge-parsley, *Torilis arvensis* (Hudson) and Corn Cleavers, *Galium tricornutum* Dandy were still recorded (Wilson, 1994).

In field and laboratory tests, broad-spectrum herbicides (e.g. glyphosate) have been found to be most damaging (Marrs *et al.*, 1991) and selective herbicides (e.g. asulam) least damaging to a range of broadleaved species (Breeze *et al.*, 1992). However, herbicides for the selective control of dicotyledons have been found to be equally as harmful as broad-spectrum herbicides to broadleaved weed and non-weed species in terms of lethal and sublethal effects of flowering and seed production suppression (Marshall & Birnie, 1985; Marrs *et al.*, 1991).

The majority of work investigating effects of herbicide on non-target arable flora has concentrated on direct applications to non-target flora, for example, crop edges where neither herbicides nor insecticides had been applied had greater plant abundance and species diversity (de Snoo, 1997). Where annual applications of glyphosate to an arable field margin were made, more annual and fewer perennial species were recorded, when compared with uncut field margins (Smith *et al.*, 1993).

Vegetation communities respond differently to broad spectrum and more selective herbicides, due to the herbicides' inherent selectivity properties. For example, Pywell *et al.* (1996) studied the effect of broad spectrum and more selective herbicides on 3 grass and 17 forb species of conservation interest and found the more selective herbicides (e.g. asulam and fluoxypyr) did not reduce the frequency of forb species to the extent of the broad spectrum herbicides (e.g. MCPA). Even though herbicides that are more selective and less destructive to non-target flora, they are still capable of altering plant communities. Although fluoxypyr, which is used for controlling annual dicotyledons, did not reduce the frequency of forb species to the same extent as less selective, broad spectrum herbicides (Pywell *et al.*, 1996), it caused a decline in species richness and forb biomass when applied at 50% recommended rate to sown grassland plots (Kleijn & Snoeijs, 1997).

Implications also exist for the non-target flora in neighbouring non-cropped habitat. De Snoo & van der Poll (1999) recorded significantly greater diversity of dicotyledons and also exclusive presence of some species (e.g. Common Poppy, *Papaver rhoeas* L.) in boundary strips adjacent to unsprayed cereal edges. However, when long-term effects of herbicide applications to crops on field margin flora at Boxworth, UK were investigated, no correlation between botanical changes and reduction in herbicide use was found (Marshall, 1987). Although this suggested that herbicide use in crops does not affect adjacent habitat, Marshall (1987) queried the possibility that previous herbicide use at Boxworth had reduced the reproductive capabilities of field margin species, thus causing the lack of correlation between botanical change and degree of herbicide use.

1.3.4 Pesticides and Non-Target Arthropods

Pesticides may affect non-target arthropods directly, indirectly, or as a combination of the two. Direct effects of pesticides arise through immediate contact with or ingestion of the active ingredient from exposure to the spray, or spray residue on soil, plant, or other surface. Indirect effects of pesticides are more complex and occur as a consequence of the direct effects of the active ingredient on i) the potential food source (e.g., reduction or contamination thereof), ii) previous life stage (e.g., sublethal effects such as reduced feeding behaviour) and, iii) through changes in microhabitat conditions (e.g., decrease in humidity).

Many factors limit the extent of pesticide exposure to individuals, and species susceptibility to pesticide is known to vary widely (Schmuck *et al.*, 1996). The hazards of agrochemicals to non-target invertebrates are related to the amount of pesticide contacted and retained by the organism, as well as the tolerance of that species to a particular

compound (Jepson *et al.*, 1990; Davis *et al.*, 1994). However, other factors such as the ecology and behaviour (Longley & Sotherton, 1997a) of the individual also play a part in determining the harmfulness of a pesticide to an organism.

Although insecticides were applied to a smaller area than other pesticides in 1996 (Table 1.1), the majority of work on pesticide impact on arthropod natural enemies until the late 1980s had been done on insecticides (82%). This contrasts with work on fungicides, acaricides and herbicides which were found to represent 9%, 7% and 1.4 % respectively of research on non-target arthropods (Theiling & Croft, 1988). Toxicity to predators and parasitoids was found to differ between types of pesticide and increased from fungicides to herbicides to insecticides (Theiling & Croft, 1988).

Research into the effects of pesticides on non-target arthropods is divided into two areas of work: laboratory toxicity experiments which test the inherent susceptibility of arthropods to pesticides and, field experiments to record any changes in abundance and diversity of arthropod species and communities following pesticide applications.

1.3.4.1 Susceptibility of Non-Target Arthropods to Pesticides

The inherent susceptibility of an organism to a pesticide is a measure of the toxic effect of the active ingredient of the pesticide, where the effect may be lethal or sublethal.

Much work has been done on toxicity testing of insecticides on non-target arthropods, with an emphasis on the synthetic pyrethroids. Table 1.2 summarises the results of insecticide toxicity testing on non-target arthropod species, indicating whether an active ingredient has been shown to be toxic and whether any sub-lethal effects were noted.

Table 1.2. Susceptibility of non-target arthropods to insecticides.

Insecticide / Arthropod	Toxic	Sublethal Effects	Author
Pyrethroid			
Deltamethrin			
<i>Pieris brassicae</i> ^L	T	reduced feeding	Çilgi & Jepson 1995
<i>Pieris rapae</i> ^L	✓	-	Çilgi & Jepson 1995
<i>Episyrphus balteatus</i> ^D	✓	-	Wiles & Jepson 1994
<i>Aphidius rhopalosiphi</i> ^H	✓	-	Wiles & Jepson 1994
<i>Agonum dorsale</i> ^C	✓	-	Wiles & Jepson 1992; Çilgi <i>et al.</i> 1996
<i>Bembidion lampros</i> ^C	✓	-	Wiles & Jepson 1992; Çilgi <i>et al.</i> 1996
<i>Bembidion obtusum</i> ^C	✓	-	Wiles & Jepson 1992/4; Çilgi <i>et al.</i> 1996
<i>Coccinella 7-punctata</i> ^C	✓	-	Wiles & Jepson 1992, 1994
<i>Demetrias atricapillus</i> ^C	×	-	Wiles & Jepson 1992/4; Çilgi <i>et al.</i> 1996
<i>Harpalus rufipes</i> ^C	✓	-	Wiles & Jepson 1992
<i>Nebria brevicollis</i> ^C	✓	rejection of sprayed aphids	Wiles & Jepson 1992, 1993, 1994
<i>Pterostichus melanarius</i> ^C	✓	-	Wiles & Jepson 1992, 1994
<i>Platymus dorsalis</i> ^C	×	-	Förster 1991
<i>Tachyporus hypnorum</i> ^C	✓	-	Wiles & Jepson 1992/4; Förster 1991
<i>Trechus quadristriatus</i> ^C	✓	-	Wiles & Jepson 1992
<i>Araneus diadematus</i> ^A	-	suppression of web-building & web size	Samu & Vollrath 1992
<i>Erigone atra</i> ^A	✓	-	Wiles & Jepson 1992
<i>Oedothorax apicatus</i> ^A	✓	reduced locomotion	Everts <i>et al.</i> 1991; Jagers op Akkerhuis <i>et al.</i> 1997
Lamda-cyhalothrin			
<i>Orius majusculus</i> ^H	✓	-	Taborsky <i>et al.</i> 1995
<i>Erigone atra</i> ^A	✓	inhibited emergence & web-building	Dinter 1996; Dinter & Poehling 1992/5
<i>Oedothorax apicatus</i> ^A	✓	delayed web-building	Dinter 1996; Dinter & Poehling 1992/5
<i>Pardosa amentata</i> ^A	✓	-	Hof <i>et al.</i> 1995
Fenvalerate			
<i>Erigone atra</i> ^A	✓	delayed web-building	Mansour <i>et al.</i> 1992; Dinter 1996
<i>Oedothorax apicatus</i> ^A	✓	delayed web-building	Mansour <i>et al.</i> 1992; Dinter 1996
<i>Pardosa agrestis</i> ^A	✓	-	Mansour <i>et al.</i> 1992
<i>Pardosa palustris</i> ^A	✓	-	Mansour <i>et al.</i> 1992
<i>Pardosa prativaga</i> ^A	✓	-	Mansour <i>et al.</i> 1992

^L: Lepidoptera; ^D: Diptera; ^H: Hymenoptera; ^C: Coleoptera; ^A: Araneae; ^H: Heteroptera
 ×: not toxic ✓: toxic; -: effect not recorded. Continued on page 15

Table 1.2. (cont).

Insecticide / Arthropod	Toxic	Sublethal Effects	Author
Organophosphorus			
Dimethoate			
<i>Pieris napi</i> ^L	×	-	Davis <i>et al.</i> 1991
<i>Agonum dorsale</i> ^C	✓	-	Çilgi <i>et al.</i> 1996
<i>Demetrias atricapillus</i> ^C	✓	-	Çilgi <i>et al.</i> 1996
<i>Bembidion lampros</i> ^C	✓	-	Hassan <i>et al.</i> , 1988; Çilgi <i>et al.</i> 1996
<i>Bembidion obtusum</i> ^C	✓	-	Çilgi <i>et al.</i> 1996
<i>Platymus dorsalis</i> ^C	✓	-	Förster 1991
<i>Tachyporus hypnorum</i> ^C	✓	-	Förster 1991
<i>Chiracanthrium milei</i> ^A	✓	-	Hassan <i>et al.</i> , 1988
Organochlorine			
Endosulfan			
<i>Argiope argentata</i> ^A	✓	-	Mansour & Nentwig 1988
<i>Chiracanthrium mildei</i> ^A	✓	-	Mansour & Nentwig 1988
<i>Erigone atra</i> ^A	✓	-	Mansour <i>et al.</i> , 1992
<i>Linyphia triangularis</i> ^A	✓	-	Mansour & Nentwig 1988
<i>Pardosa agrestis</i> ^A	✓	-	Mansour <i>et al.</i> 1992
<i>Pardosa palustris</i> ^A	✓	-	Mansour <i>et al.</i> 1992
<i>Pardosa prativaga</i> ^A	✓	-	Mansour <i>et al.</i> 1992
Carbamate			
Pirimicarb			
<i>Calocoris norvegicus</i> ^{Ht}	×	-	Moreby 1994
<i>Agonum dorsale</i> ^C	×	-	Çilgi <i>et al.</i> 1996
<i>Bembidion lampros</i> ^C	×	-	Çilgi <i>et al.</i> 1996
<i>Bembidion obtusum</i> ^C	slight	-	Çilgi <i>et al.</i> 1996
<i>Demetrias atricapillus</i> ^C	×	-	Çilgi <i>et al.</i> 1996
<i>Argiope argentata</i> ^A	×	-	Mansour & Nentwig 1988
<i>Chiracanthrium mildei</i> ^A	×	-	Mansour & Nentwig 1988
<i>Erigone atra</i> ^A	×	-	Dinter & Poehling 1995
<i>Linyphia triangularis</i> ^A	slight	-	Mansour & Nentwig 1988
<i>Oedothorax apicatus</i> ^A	×	-	Dinter & Poehling 1995
<i>Philodromus aurelous</i> ^A	×	-	Mansour & Nentwig 1988

^L: Lepidoptera, ^C: Coleoptera; ^A: Araneae; ^{Ht}: Heteroptera

×: not toxic ✓: toxic; -: effect not recorded

Pirimicarb, a selective systemic carbamate insecticide used to control aphids (Tomlin 1994), has consistently been shown to be the least toxic of the insecticides tested, with many species exhibiting degrees of tolerance (Table 1.2). Çilgi *et al.*, (1996) tested the residual toxicity of pirimicarb, deltamethrin and dimethoate to four species of Carabidae and recorded that pirimicarb was the least toxic reaching a maximum of 95% mortality for the most susceptible species, *Bembidion obtusum* (Serville) at 100% of the highest recommended field rate.

The synthetic pyrethroid, organophosphorous and organochlorine insecticides are more toxic to non-target arthropods than the carbamates. The broad-spectrum, non-systemic pyrethroid insecticides have been shown to be toxic to many arthropods and are tolerated by just three species (two carabid beetles and the larvae of one species of butterfly) (Table 1.2).

Laboratory screening of pesticides other than insecticides on non-target arthropods has been much less prolific. Moreby (1991) tested the toxicity of 8 fungicides to nymphs of *Calocoris norvegicus* (Heteroptera: Miridae) and found that tridemorph and fenpropimorph had insecticidal properties. The toxicity of herbicides to non-target arthropods has also been shown to be low. Brust (1990) tested the residual toxicity of the herbicides atrazine, simazine, paraquat and glyphosate to a group of Carabidae and found no differences in mortality between carabids exposed to treated and untreated soil. Moreby (1991) tested 9 herbicides on *Calocoris norvegicus* nymphs and found that only fluoxypyr had significant insecticidal properties.

Although some pesticides may have been identified as being toxic to certain species, susceptibility of arthropods to a pesticide is not always consistent across taxa. For

example, the carabid beetles *Demetrias atricapillus*, *Bembidion obtusum* and *B. lampros* (Herbst) were found to be susceptible to dimethoate (Çilgi *et al.*, 1996), an organophosphorus broad-spectrum, systemic insecticide and (Tomlin, 1994). However, *Pieris napi* (L) larvae (Lepidoptera: Pieridae) were tolerant of dimethoate (Davis *et al.*, 1991). Furthermore, Sinha *et al.* (1990) note that per unit weight dimethoate is 6200 times more toxic to *Apis mellifera* L. (Hymenoptera: Apidae) than to *Pieris brassicae* (L) (Lepidoptera: Pieridae) larvae.

Susceptibility to insecticides can also vary between species within the same taxon. For example, in the order Araneae, *Pardosa* species (Lycosidae) are more susceptible than *Erigone atra* (Blackwall) (Linyphiidae) (Mansour *et al.*, 1992) to fenvalerate, whilst within the family Linyphiidae (Araneae), *E. atra* is more susceptible than *Oedothorax apicatus* (Blackwall) (Dinter & Poehling, 1995). Thus, accurate interpretation of susceptibility of untested arthropod species to pesticides from a limited number of test species is not possible.

Differences in susceptibility to pesticides related to age-class and sex have been observed. Juvenile *Erigone atra* and *Oedothorax apicatus* are more susceptible than adult females to the pyrethroid insecticides lambda-cyhalothrin and fenvalerate (Dinter, 1996), and the males are more susceptible than females (Dinter & Poehling, 1995).

While many toxicity experiments have been concerned with mortality rates, some authors have studied the sublethal effects of pesticides on non-target arthropods that may be manifest in both current and subsequent generations of arthropod. Chiverton & Sotherton (1991) found that where herbicides had been excluded from field edges, female *Pterostichus melanarius* and *Agonum dorsale* produced higher numbers of eggs, and

concluded that herbicide applications could reduce the level of carabid beetle fecundity. Other sublethal effects include changes in behaviour. Deltamethrin, for example, reduced walking speed in *Oedothorax apicatus*, which resulted in an increased rate of predation by carabid beetles (Everts *et al.*, 1991). Samu *et al.* (1992) found that *Araneus diadematus* Clerck (Araneae: Araneidae) webs effectively collected pyrethroid insecticide spray drift which was then shown to result in suppression of web-building frequency and size (Samu & Vollrath, 1992).

Effects of sublethal doses of some insecticides to arthropods have been shown to affect development to the next developmental stage in their life history. The pyrethroid insecticide, deltamethrin, inhibited feeding behaviour in *Pieris brassicae* larvae, that went on to produce smaller pupae and adults (Çilgi & Jepson, 1995). Since the size of an individual is one of the components of ecological fitness that influences mating success in insects (Markow & Ricker, 1992), deltamethrin may influence the lifetime mating success of the *P. brassicae* and possibly other arthropods that exhibit reduced feeding activity. Lambda-cyhalothrin, another pyrethroid insecticide, inhibited the emergence of *Erigone atra* spiderlings from egg sacs (Dinter, 1996) and delayed the web-building activity of *E. atra* and *O. apicatus* (Dinter & Poehling, 1995), where the latter effect of the insecticide has implications for prey capture and ecological fitness of the spiders.

Consumption of pesticide-contaminated food items can also influence mortality in arthropods. Thacker & Hickman (1990) sprayed aphids with the fungicide pyrazaphos and fed them to the carabid beetle *Agonum dorsale* (Ponoppidan) and found that when only treated aphids were consumed, mortality in *A. dorsale* was 100%.

1.3.4.2 Effect of Field Applications of Pesticides on Non-Target Arthropods

Field-based experiments have studied the effects of pesticide applications to cropped and non-cropped areas on non-target arthropods, but have not examined the toxic effects. Therefore, these types of investigation have only studied changes in relative abundance and diversity of non-target arthropods where pesticides have been applied.

Monitoring the effects of pesticide applications to cropped areas on non-target arthropods has been widespread, with the majority of field experiments concentrating on the impact of insecticides. The first large-scale, long-term experiment to investigate the environmental effects of pesticides in the UK was the Boxworth Project. The project was initiated in the late 1970s (Greig-Smith, 1991) and ran for 7 years (Frampton, 1998). The project compared three approaches to crop protection: i) prophylactic, high-input; ii) planned reduced-input approach, where pesticide application was measured against pest, weed and disease levels and, iii) integrated pest management, including the use of specific pesticides and disease resistant varieties (Greig-Smith & Hardy, 1992). The project found that compared with the reduced input areas (approaches ii and iii), abundances of Collembola and the carabid beetles *Bembidion aenum* (Germar), *B. lunulatum* (Geoffrey-Fourcroy), *B. obtusum*, *Trechus quadristriatus* (Schränk) and *Nebria brevicollis* Fab. (larvae) in the intensive areas were significantly reduced subsequent to applications of chlorpyrifos (Frampton & Çilgi, 1992). *Erigone* spiders and *Helophorus* beetles were significantly reduced by chlorpyrifos and deltamethrin insecticides (Frampton & Çilgi, 1992).

Other field experiments have been smaller in scale and short term, often comparing the arthropod fauna in treated areas with untreated areas over one season. Most authors have studied the effects of insecticides on beneficial arthropods, especially on the Araneae and

Carabidae. The pyrethroid and organophosphorus insecticides are the most harmful to the abundance of non-target arthropods. Spider densities in arable fields were significantly reduced by pyrethroid (Brown *et al.*, 1988; Everts *et al.*, 1989; Thomas *et al.*, 1990; Jagers op Akkerhuis, 1993; Thomas & Jepson, 1997) and organophosphorus (Everts *et al.*, 1989; Thomas & Jepson, 1997) insecticides. Carabid beetles were similarly affected being significantly reduced by applications of pyrethroid (Brown *et al.*, 1988; Everts *et al.*, 1989; Curtis & Horne, 1995) and organophosphorus (Edwards *et al.*, 1979; Everts *et al.*, 1989; Curtis & Horne, 1995) insecticides. Other groups of invertebrate are also sensitive to the effects of pesticide: Aebischer (1990) found that dimethoate significantly reduced sawfly densities in fully sprayed cereal fields. In concurrence with toxicity testing of pirimicarb on non-target arthropods, field applications of the insecticide did not result in reduced abundance of Heteroptera (Moreby *et al.*, 1997), providing further evidence that pirimicarb is the least harmful insecticide to populations of non-target arthropods.

The effect of field applications of fungicides on non-target arthropods has had limited attention, but some differences in response to various active ingredients have been identified. Pyrazaphos applied at the recommended field rate significantly reduced aphid natural enemies (Carabidae and Staphylinidae), Collembola and gamebird chick food items including the Heteroptera (Sotherton *et al.*, 1987). However, tridemorph, propiconazole and prochloraz did not significantly reduce Heteroptera in a headland of winter wheat (Moreby *et al.*, 1997).

Studies have examined the effect of herbicide applications to cropped and non-cropped areas on non-target arthropods. One of the earliest studies of the effects of herbicide applications to arable crops on non-target arthropods was done by Raatikainen & Huhta (1968) who recorded significantly fewer spiders and species of spider in herbicide treated

oats. Chiverton & Sotherton (1991) observed that unsprayed headlands supported more non-target arthropods, especially non-pest species and more predatory arthropod groups than sprayed headlands. Also, applications of the herbicides MCPB, MCPA and 2, 4-DP + MCPA to barley significantly reduced arthropod biomass (Southwood & Cross, 1969).

The response of some arthropod groups to different herbicides has been shown to vary: Brust (1990), for example, found that the broadspectrum herbicides paraquat and glyphosate significantly reduced carabid beetle abundance in winter wheat, whereas applications of MCPA/MCPB did not have the same effect (Everts *et al.*, 1989). Furthermore, responses to individual herbicides by arthropods are known to vary between taxonomic groups. For example, although MCPA and MCPB reduced arthropod biomass in barley (Southwood & Cross, 1969), Everts *et al.* (1989) found that applications of these herbicides to winter wheat did not affect carabid beetle or spider abundance.

The response of Heteroptera to herbicide applications has been shown to be consistent, since their abundance (Chiverton & Sotherton, 1991) and diversity (Moreby & Southway, 1999) were significantly greater in unsprayed headlands than in herbicide sprayed headlands. Research into the effects of herbicide applications to non-cropped areas is scant. However, a project examining methods of field margin management for nature conservation found that a single field rate application of glyphosate to a field margin reduced the abundance of Linyphiidae (Araneae), but not of the Staphylinidae (Coleoptera) (Feber *et al.*, 1995).

General studies examining the effect of pesticide applications to cropped areas *per se* on non-target arthropods have largely focused on the Lepidoptera. Butterflies were more abundant in selectively sprayed conservation headlands than in fully sprayed headlands

(Dover *et al.*, 1990), in unsprayed headlands than sprayed headlands (Rands & Sotherton, 1986) and in winter wheat sprayed with fungicide only than in conventional managed winter wheat (de Snoo, 1994). Not only were butterflies more abundant in reduced pesticide input areas, but their diversity also increased (Rands & Sotherton, 1986). The reduction in butterfly abundance and diversity associated with agrochemical input is known to be related to the reduced availability of nectar and foodplant resources where herbicides have been used (Dover *et al.*, 1990; Feber *et al.*, 1996).

Groups of arthropod other than Lepidoptera have also been shown to be affected by pesticide use. Selectively sprayed headlands supported greater numbers of hunting spiders (Lycosidae) than conventionally managed ones (Hassall *et al.*, 1992; White & Hassall, 1994) and unsprayed crop margins have been shown to support greater abundances of gamebird chick prey items (Sotherton *et al.*, 1985; Chiverton, 1999).

The type of farming system also affects arthropod abundance, especially those that employ either an integrated pest management regime where pesticides are applied when necessary, or an organic policy where pesticide use is not permitted. When organic and conventional farming systems were compared, it was found that there was a greater abundance and diversity of spiders (Feber *et al.*, 1998), more Collembola (Moreby *et al.*, 1994) and non-pest butterfly species (Feber *et al.*, 1997) in organic farms. Much of the variation between arthropod abundance and diversity in organic and conventional farming systems can be explained by increased weediness within the crop (Feber *et al.*, 1998).

Although non-target plants and arthropods are affected by direct applications of pesticide to the cropped habitat in the agro-ecosystem, misplacement of pesticide to non-cropped areas also poses a potential threat, particularly in the form of spray drift.

1.4 Spray Drift

Although pesticides are applied in a number of ways, approximately 75% of all pesticides are applied as sprays (Cremlyn, 1991). In the UK, 90% of all sprays are applied using conventional hydraulic sprayers (Davis & Williams, 1993). On a world-wide scale, less than 0.1% of the 5×10^6 billion tonnes of pesticide applied to crops actually reaches the target (Albert *et al.*, 1992), thus the misplacement of pesticides is a considerable problem. Indeed, it is estimated that spray drift could account for a maximum of one third of the spray volume applied to a target area (Merritt, 1989).

1.4.1 Causes and Mechanisms of Spray Drift

Spray drift is the aerial transport of pesticide away from the target directly subsequent to application (Cooke, 1993) by the action of the wind (Miller, 1993). The physical mechanics behind spray drift are extremely complex. Pesticide drift occurs as droplets of spray solutions or vapour (Breeze *et al.*, 1992). Furthermore, the extent of spray drift is influenced by operator variables (e.g. equipment, nozzles, operating pressure and release height), meteorological factors (e.g. wind-speed, atmospheric conditions, temperature and relative humidity), and site variables (e.g. field size, crop, hedges and landform) (Elliott & Wilson, 1983; Davis & Williams, 1993). These influencing factors interact in a dynamic manner to create spray dispersal and drift.

The influence of droplet size is an important determinant of the potential for spray drift. Large droplets of spray applied at close distances reach their target quicker than smaller droplets because they have greater momentum, and since they have a smaller surface area relative to volume, evaporation is slower (Davis & Williams, 1993). Therefore, the

finer the spray quality, the more likely an increase in potential for a spray drift event to occur. Simplified, spray drift is essentially the result of i) small spray droplets being discharged from the spray nozzle, ii) pesticide being applied at some distance from the target and iii) strong wind moving the spray deposits away from the target, or a temperature inversion causing the droplets to fall more slowly and volatilising into more mobile droplets (Mueller & Womac, 1997).

1.4.2 Methods of Spray Drift Reduction

Since there are many contributing factors to the occurrence of a spray drift event, there are numerous options for managing its reduction.

Firstly, machinery variables may be manipulated to reduce the incidence of spray drift. Techniques include the use of rotary atomisers with controlled droplet application (CDA) capability, low drift nozzles, air assistance and ensuring the boom is set at an appropriate height above the target area. CDA reduces the output of the small droplets that are prone to drift (Holland *et al.*, 1997). However, the non-CDA hydraulic nozzles are often cheaper than the more expensive technology (Holland *et al.*, 1997) and therefore their use is likely to be more widespread. The type of tank mix may also assist in the reduction of spray drift. Adjuvants are often added to pesticide formulations to enhance their biological activity. It has been recorded that vegetable oil adjuvants can reduce the proportion of small droplets, which in turn reduces spray drift (Western *et al.*, 1999).

Secondly, guidelines for appropriate spraying conditions are determined by wind speeds (Anon, 1998) since spray drift events are more likely occur in higher wind speeds than lower ones (Davis *et al.*, 1993b). Therefore, spraying at lower wind speeds reduces the

likelihood of a drift event.

Thirdly, site variables may be used as a means of reducing spray drift. Hedgerows reduce air turbulence at field edges (Lewis, 1965), and therefore reduce the risk of spray drift. Increasing the distance from the sprayer also reduces the opportunity for agrochemical drift into non-crop, sensitive areas and an ideal method of achieving this is the use of buffer strips.

Finally, the Local Environmental Risk Assessment for Pesticides (LERAP) scheme, which aims to combine the three preceding methods of reducing spray drift while taking into account the toxicity of the pesticide to be applied, has recently been adopted (Anon, 1999a). LERAPs aim to simplify and make more enforceable the existing control of pesticides legislation (Croxford, 1998), however they are targeted towards areas adjacent to watercourses (Anon, 1999a) and do not consider the ecological value of the non-aquatic environment.

Much work has been done on measuring spray drift by recording the deposition of drift on passive collectors, such as pipecleaners and haircurlers. Spray drift is significantly reduced with increasing distance from the sprayer (Cuthbertson, 1988; Davis *et al.* 1993c; Tooby, 1997), while hedges have been shown to act as interceptors of drift (Davis *et al.*, 1993c; Rautmann *et al.*, 1997). Unsprayed headlands, which tend to be 6m wide, are also effective in reducing spray drift into hedgerow and field margin areas and have reduced drift from between 70% (Cuthbertson & Jepson, 1988) and 100% at low wind speeds (Çilgi, 1993).

1.4.3 Measuring Spray Drift

Pesticide sprays have been recorded by researchers for many years in order to determine their fate. An understanding of the efficiency and likely effectiveness of application techniques is acquired through knowledge of the fate of pesticides applied for crop protection (Cooke & Hislop, 1993).

Spray drift may be measured using a variety of methods, which range in efficiency, ease of use and expense. Five main methods of drift collection exist and are described below:

- 1) Volumetric air samplers are efficient collectors and draw air over a filtering medium to quantify the spray captured, however, this method is very expensive and complex (Miller, 1993);
- 2) Rotary samplers rotate collection surfaces about a vertical central axis, but this generates air flow which alters the sampling volume (Miller, 1993);
- 3) Passive surface collectors collect airborne droplets on static targets, by a process of impaction (Miller, 1993). It is important that the target collects a representative sample of drifting spray;
- 4) Laser-based sampling systems measure airborne droplet fluxes and are useful for laboratory tests, however they are very expensive and complex (Miller, 1993); and,
- 5) Plant surfaces as indicators of drift record the effects of pesticides, however, toxic vapours and not drift may affect plants, giving erroneous results (Miller, 1993).

Spray tracers identify the course of drift by marking the spray droplets and many techniques for this process exist (Cooke & Hislop, 1993). The most commonly used techniques are those involving the use of visible and fluorescent dyes since they are both

inexpensive and effective when used appropriately (e.g. Sharp, 1974; Cooke & Hislop, 1993; Cross *et al.*, 1997). Visible dyes require measurement of the colour components at specific wavelengths of colour absorption in an extraction fluid (Cooke & Hislop, 1993). Fluorescent dyes are extracted and fluoresced under ultra-violet light (Sharp, 1974) and are detectable even at low concentrations (Cooke & Hislop, 1993), making them more sensitive than the visible dyes. However, the main disadvantage of some fluorescent dyes such as fluorescein, is that they are photosensitive and samples must be placed in a lightproof container within 15 minutes of spray application (Sharp, 1974; Cooke & Hislop, 1993).

1.4.4 Effects of Spray Drift on Non-Target Biota

The effects of spray drift on non-target biota frequently occur, and events involving great amounts of herbicide drift are easy to identify where vegetation adjacent to the crop shows signs of damage (Plate 1.1). Much work on the impact of pesticide drift has been carried out by the Institute of Terrestrial Ecology for the Nature Conservancy Council and Department of Environment in the late 1980s (Davis, 1992). However, studies on the effect of herbicide drift on non-target plants and insecticide drift on non-target invertebrates have dominated the research on environmental impacts of spray drift.



Plate 1.1 Effect of herbicide drift on non-cropped area. Cheney Hill, Newport, Shropshire. (SJ738199)

Herbicide drift impacts upon non-crop vegetation adjacent to sprayed areas and may have lethal or sublethal effects. When studying the effects of herbicide drift on a selection of plant species, Marrs *et al.* (1989a) found that the maximum safe distance from the sprayer for protection against lethal effects of herbicides (glyphosate) was 5m, but for most species 2m provided adequate protection. When safe distances for protection against any visible damaging effects of herbicide drift were investigated, it was found that some species were sensitive up to 20m away from the sprayer (Marrs *et al.*, 1989b).

Potential effects of herbicide drift may be determined by the age of plants, since younger plants tend to be more sensitive to herbicide than older plants (Marrs *et al.*, 1991). Marrs *et al.*, (1992a), for example, note that established perennials may avoid herbicide damage at distances up to 10m away from the sprayer, while for establishing seedlings, the required distance increases to 20m. Surrounding vegetation is also known to affect the impact of herbicide drift on non-crop flora, although interpretation of the implications to individual species is difficult and complex (Marrs *et al.*, 1991).

Whilst these studies have examined the effects of herbicide drift on individual plant species, more general studies have examined the effect of herbicide drift on plant communities. The diversity and cover of dicotyledons in ditch bank vegetation adjacent to winter wheat was significantly lower where the crop had been sprayed with herbicide (de Snoo & van der Poll, 1999). In an experiment that simulated herbicide spray drift, a decline in species richness was observed and the biomass of spontaneously colonising forbs was decreased (Kleijn & Snoeiijing, 1997).

Studies examining the environmental impacts of pesticide drift have also included work on insecticides and insects, paying particular attention to some Lepidoptera larvae. When insecticide spray drift into field margin and hedgerow habitat was examined, Davis *et al.* (1991) found that exposure of Lepidoptera larvae proved fatal at up to 16m from sprayer. Using toxicity and drift data, it was predicted that deltamethrin would cause high levels of mortality of 4th instar *Pieris brassicae* (Lepidoptera: Pieridae) in hedgerows adjacent to fully sprayed headlands (Çilgi & Jepson, 1995). More direct evidence also exists which indicates that insects adjacent to sprayed areas are at risk. Longley & Sotherton (1997b) found that *Spodoptera littoralis* (Lepidoptera: Noctuidae) and *P. brassicae* larvae suffered high mortality rates when exposed to residual amounts of insecticide on grasses that were

adjacent to a fully sprayed headland. Dimethoate drift at ground level in a hedgerow was 0.5% of the field application rate causing approximately 90% mortality and knockdown in *Demetrias atricapillus*, *Bembidion lampros* and *B. obtusum* (Coleoptera: Carabidae) (Çilgi *et al.*, 1994).

Distance from the sprayer can reduce insect mortality rates from insecticide drift. In an experiment measuring the mortality of carabid beetles caused by insecticide drift, it was found that dimethoate drift at ground level 4 m away from the sprayed area was less than 0.2% of the field rate and significantly reduced mortality of *D. atricapillus*, *B. lampros* and *B. obtusum* compared with spray application adjacent to the hedgerow (Çilgi *et al.*, 1994). The mortality of *P. brassicae* caused by drift of the insecticide cypermethrin was significantly reduced 5 m downwind of the sprayer, and was further reduced if a hedge was present between the sprayer and the insect (Davis *et al.*, 1994). The presence of a hedge between the sprayer and non-target area reduces the spray drift into the non-target area (Davis *et al.*, 1994), because barriers, such as hedges and fences, reduce wind speed both to the leeward and the windward (Lewis, 1965).

1.5 Glyphosate

Glyphosate, a non-selective, systemic herbicide absorbed by foliage and translocated throughout the plant, is used to control annual, biennial and perennial dicotyledons, grasses and sedges (Tomlin, 1994; Kidd & Casely, 1999). The herbicide is used for pre-emergence and pre-harvest weed control in cereals and some other crops; pre-harvest desiccation in cotton, cereals and oil seed rape; destruction of grassland; general weed control in non-cropped habitats and, for aquatic weed control (Tomlin, 1994; Whitehead, 1997; Anon, 1999b).

Since its introduction in 1974, glyphosate has become one of the most heavily used herbicides, largely due to its versatility, low environmental toxicity (e.g., Hassan *et al.*, 1988) and the large number of world-wide generic producers since the recent expiry of the patent (except in the US) (Kidd & Casely, 1999) ensuring the continued fall in price (Francesca Tencalla, pers. comm., Monsanto, Louvain-La-Neuve, Belgium, 1999).

In the UK, glyphosate use on arable farm crops (excluding set-aside) increased dramatically and rose from an average of 262.71 tonnes a.i. over the period 1992 - 1996 (Davis *et al.*, 1993; Garthwaite *et al.*, 1995; Thomas *et al.*, 1997) to 634.76 tonnes a.i. in 1998 (D. Garthwaite, pers. comm., Pesticide Usage Survey Group, Central Science Laboratories, York, 1999). Glyphosate is probably the most commonly-used herbicide in set-aside, with its use accounting for 17% of all glyphosate applications to farmland in 1998 (D. Garthwaite pers. comm.). Set-aside covers large areas of farmland in UK and continental Europe and with rotational set-aside, different areas of non-cropped habitat, including field margins, can be exposed to glyphosate on an annual basis when considered in the long-term. Glyphosate use has also increased in the US where glyphosate-tolerant crops are now being cultivated (Kidd & Casely, 1999), and if herbicide tolerant crops are licensed to be grown in Europe, a similar further increase in UK glyphosate use is also likely.

It is the change from the more 'traditional' autumn applications of glyphosate to spring application (when vegetation is actively growing and uptake is therefore more efficient), particularly if permission is granted for the cultivation of genetically modified herbicide resistant crops, that raises the issue of the effect of herbicide drift into arable field margins on the non-target arthropod fauna.

Studying the effects of pesticides on non-target arthropods is a complex and multifaceted exercise, and Jepson (1989) devised a theoretical framework of the chronological sequence of processes of environmental contamination leading to the effects of pesticides on non-target arthropods within an arable crop, recommending appropriate scales at which investigations should occur. Figure 2.1 illustrates this process of contamination caused by a pesticide and approaches based on these processes were adopted to investigate the likely effects of herbicide drift into grassy arable field margins on flora and Araneae, Carabidae and Heteroptera.

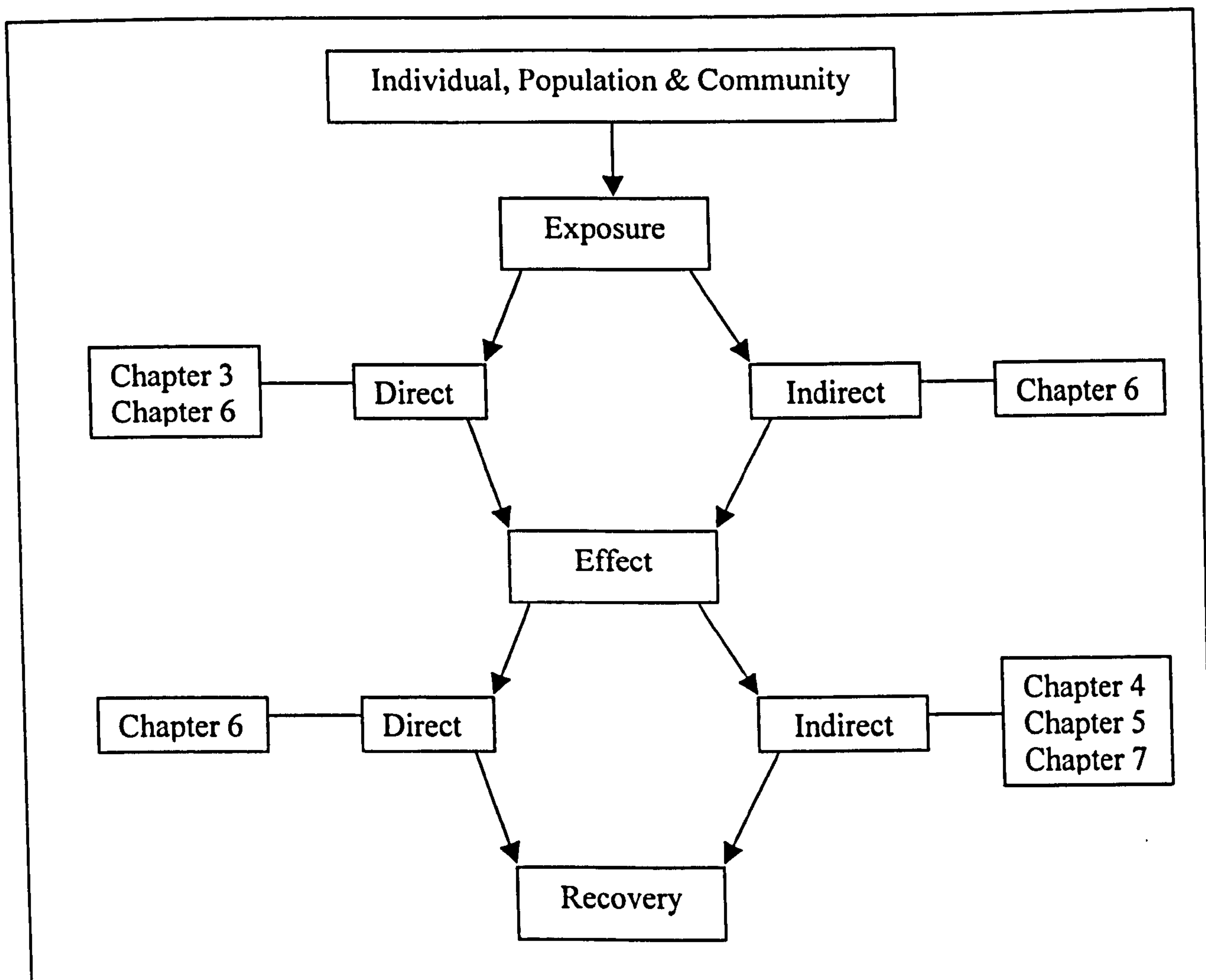


Figure 2.1. Chronological sequence of the process of environmental contamination leading to the effects of a pesticide on non-target arthropods within an arable crop (Adapted from Jepson, 1989).

Thus, this study aims determine likely effects of glyphosate spray drift into grassy arable field margins on non-target arthropods (Araneae, Carabidae and Heteroptera) by:

- i) Quantifying patterns of spray drift intercepted by plant species in fully sprayed field margins and those protected by buffer strips, in order to determine how rapidly drift is reduced and whether interspecific differences in interception exist (Chapter 3);
- ii) Measuring the dose response of plant species to rates of glyphosate that simulate rates of active ingredient under spray drift conditions (Chapter 4);

- iii) Determining whether glyphosate applications to food plants affect dependent herbivores (Chapter 5);
- iv) Testing whether glyphosate is toxic to non-target arthropods (Chapter 6); and,
- v) Measuring the response of non-target arthropods to applications of glyphosate to grassy field margins that are representative of field and drift rates of active ingredient (Chapter 7).

3 SPRAY DRIFT INTERCEPTION BY PLANTS IN DIFFERENT WIDTHS OF BUFFER STRIP

3.1 Introduction

Spray drift is the movement of spray droplets away from the target (Cooke, 1993) and is a complex phenomenon. Spray drift is dependent on many factors, including wind velocity, air humidity, temperature (Elliott & Wilson, 1983; Miller, 1993; Nordbo *et al.*, 1993) and the equipment dispensing the spray (Miller, 1993; Holland *et al.*, 1997). However, spray drift is essentially the result of small spray droplets being discharged from a nozzle at a great distance from the target and wind and/or temperature inversions causing spray droplets to be displaced away from the target (Mueller & Womac, 1997). One method of reducing spray drift into non-target areas is to increase the distance from the sprayer to the non-target area by using buffer strips.

Buffer strips have been shown to be effective in reducing spray drift on non-target organisms (e.g. Marrs *et al.*, 1992a; Davis *et al.*, 1993b). The measurement of spray drift at distances downwind of the sprayer has generally relied on using artificial receptors, such as pipecleaners, wool and haircurlers as the targets (Çilgi & Jepson, 1992; Çilgi 1993; Davis *et al.*, 1993c; Nordbo *et al.*, 1993; Perry *et al.*, 1996; Western *et al.*, 1999) and a fluorescent dye as the tracer (Cuthbertson & Jepson, 1988; Çilgi & Jepson, 1992; Çilgi, 1993; Cross *et al.*, 1997; Miller & Lane, 1999), however, while artificial receptors give an estimate of the amount of spray drift reaching an object, they do not provide any indication of possible variance in drift interception susceptibilities between species.

This experiment aimed to evaluate the effectiveness of three widths of buffer strip in reducing the amount of spray drift deposited on different plant species adjacent to these strips. The effects of plant height, leaf texture, shape and size on drift interception were examined. The three distances chosen were 0m (fully sprayed), 2m and 6m from the sprayer, where the fully sprayed strip represents no active protection from exposure to spray drift and is the most common current scenario in UK arable farmland, while the 2m and 6m wide buffer strips are analogous to the grass field margins and beetle banks option in the Countryside Stewardship Scheme (MAFF, 1996) and conservation headland option in the pilot Arable Stewardship Scheme (MAFF, 1998).

3.2 Materials & Methods

Sixteen species of grasses and forbs commonly found in grassy arable field margin communities were selected to represent a range of leaf shapes, sizes and texture (Table 3.1). Leaf shape was classified as long, oval, divided or pinnate and leaf texture was identified as either hairy or non-hairy. Leaf size given in Table 3.1 is summarised from leaf area measurements used in the fluorimetry analyses.

Fifteen individual plants of each species were raised from native seed in late January 1997 using John Innes No.2 compost in 10cm pots. Seedlings were weeded and kept in a frost-free polytunnel until the possibility of severe frosts had passed, when they were moved to an external, enclosed rabbit-proof holding area. The aim of keeping the plants as frost-free as possible was to minimise loss of individuals from the experiment.

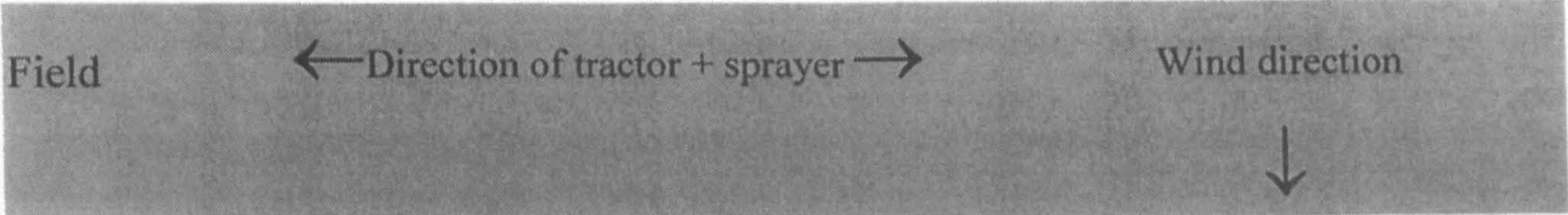
Table 3.1. Plant species selected for measuring spray drift into different widths of buffer strip and their leaf area, shape and texture properties. Leaf areas are based on measurements of leaves used in fluorimetry analyses.

Latin Binomial	Common Name	Leaf Characteristics		
		Area	Shape	Texture
<i>Silene alba</i> (Sa)	White campion	medium	oval	hairy
<i>Stellaria media</i> (Sm)	Common chickweed	small	oval	non-hairy
<i>Cerastium holosteoides</i> (Ch)	Common mouse-ear	small	oval	hairy
<i>Geranium robertianum</i> (Gr)	Herb-Robert	medium	divided	hairy
<i>Rumex obtusifolius</i> (Ro)	Broad-leaved dock	large	oval	non-hairy
<i>Lamium album</i> (La)	White dead-nettle	medium	oval	hairy
<i>Tripleurospermum maritimum</i> (Tm)	Scentless mayweed	small	divided	non-hairy
<i>Cirsium vulgare</i> (Cv)	Spear thistle	large	pinnate	hairy
<i>Cirsium arvense</i> (Ca)	Creeping thistle	large	pinnate	non-hairy
<i>Centaurea nigra</i> (Cn)	Black knapweed	medium	long	hairy
<i>Elymus repens</i> (Er)	Couch grass	medium	long	non-hairy
<i>Festuca rubra</i> (Fr)	Red fescue	small	long	non-hairy
<i>Lolium perenne</i> (Lp)	Perennial rye-grass	small	long	non-hairy
<i>Dactylis glomerata</i> (Dg)	Cocks foot	large	long	non-hairy
<i>Arrhenatherum elatius</i> (Ae)	Oat grass	large	long	non-hairy
<i>Agrostis stolonifera</i> (As)	Creeping bent	small	long	non-hairy

Nomenclature After Stace (1997). Letters in parentheses are abbreviations of plant species names.

The experiment was replicated 5 times, where each replicate took place on a different day. This was due to both the amount of time taken to process the samples and the importance of avoiding significant degradation of the sodium fluorescein through lengthy exposure to ultra-violet light. Prior to spraying, each plant species was assigned a random number, which indicated its position in each buffer strip: this position was maintained for each replication of the experiment (Figure 3.1). On the day of spraying (17.vii – 29.vii.1997), one plant of each species was placed in its allotted position adjacent to each buffer strip, where the distance

Sodium fluoride (Harris Laboratory Supplies, Birmingham, UK) 100 g to each with
 positioned perpendicular to the wind direction in rough grassland at Harper Adams University
 College, Shropshire (SJ 712204). Plate 3.1 shows the experiment in progress.



Fully Sprayed	<u>Ae Ca Er Sa La Ch Ro Fr Cn Dg Tm Cv Lp Gr Sm As</u>
---------------	--------------------------------------------------------

2m buffer strip	<u>Ae Ca Er Sa La Ch Ro Fr Cn Dg Tm Cv Lp Gr Sm As</u>
-----------------	--------------------------------------------------------

6m buffer strip	<u>Ae Ca Er Sa La Ch Ro Fr Cn Dg Tm Cv Lp Gr Sm As</u>
-----------------	--------------------------------------------------------

Figure 3.1. Configuration of the 16 plant species in the fully sprayed, 2m and 6m wide buffer strips for each replicate of the drift experiment. Refer to Table 3.1 for key to abbreviations.

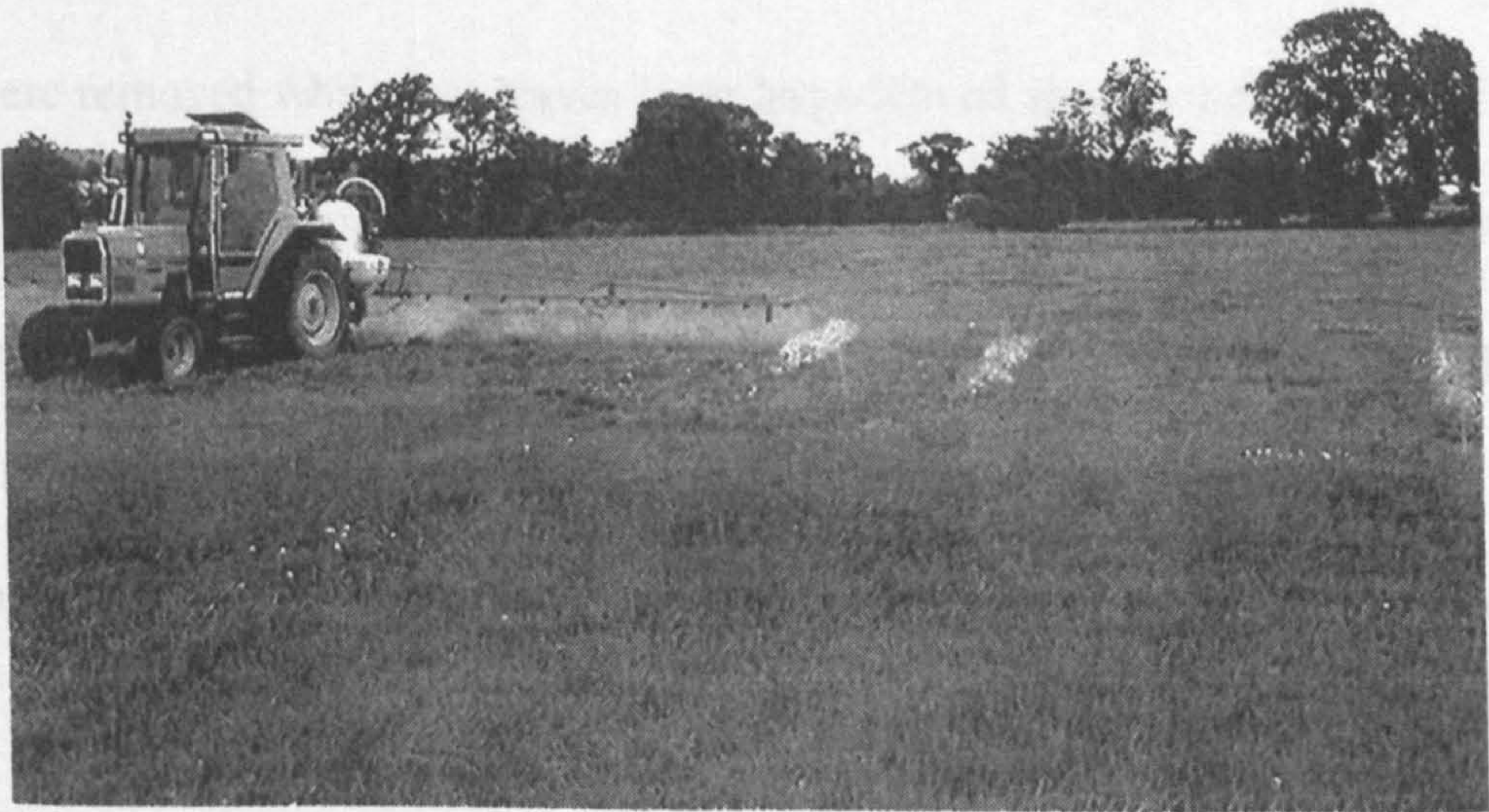


Plate 3.1 Drift experiment in progress

Sodium fluorescein (Hogg Laboratory Supplies, Birmingham, UK) (1g in 10 litres water + 0.1% v.v. Agral (Zeneca, Fernhurst, Surrey, UK)) was applied at a volume rate of 265 litres ha⁻¹ from a Hardi LX600 sprayer fitted with Hardi 4110-20 nozzles at spray pressure of 3 bar and a tractor forward speed of 2m s⁻¹. The 12m boom was positioned 50cm above tallest plant height and 4 double passes of the buffer strips were made to ensure detectable levels of fluorescein were present during fluorimetry analysis. Wind speed during each application was recorded using a Testo 440 Anemometer and always measured less than 3m s⁻¹ (Table 3.2).

Table 3.2. Mean wind speeds for each replicate of the spray drift experiment.

Replicate	Wind Speed (m s ⁻¹)
1	1.95
2	2.24
3	1.21
4	1.94
5	2.20

Leaves were removed from the lower parts of each plant before spraying commenced to obtain a measurement of natural fluorescence. To ensure that adequate amounts of sodium fluorescein were intercepted by smaller leaved plant species, four leaves from small-leaved species were removed while two leaves from large-leaved species were taken (Table 3.3). This does not have an impact on the results since measurements were in $\mu\text{l deposit mm}^{-2}$ of leaf. The leaves from each plant were placed in plastic bags, labelled and then placed in a large, light-proof bag. Immediately after spraying had finished, leaf samples were taken from each plant and stored as before in the light-proof bag, except this time leader leaves were removed. The light-proof bag was used to limit deterioration of fluorescence of the sodium fluorescein in ultra-violet light. The time taken from the end of fluorescein spraying to placing

the last bag of sprayed leaves in the black plastic bag was less than 30 minutes.

Table 3.3. Plant species that had four leaves removed in the spray drift experiment.

Latin Binomial	Common Name
<i>Stellaria media</i>	Common chickweed
<i>Cerastium holosteoides</i>	Common mouse-ear
<i>Tripleurospermum maritimum</i>	Scentless mayweed
<i>Festuca rubra</i>	Red fescue
<i>Lolium perenne</i>	Perennial rye-grass
<i>Agrostis stolonifera</i>	Creeping bent

Nomenclature After Stace (1997)

All leaves were washed in a standard non-ionic buffer solution (50ml water + 0.1% v.v Agral) and the fluorescence concentration in this solution was analysed using a Perkin Elmer luminescence spectrophotometer LS30 (excitation wavelength 420nm, emission wavelength 508nm). The difference in fluorescence between the unsprayed leaves (natural fluorescence) and leaves exposed to spray (natural + tracer fluorescence) was the amount of spray intercepted by the leaves and was measured in μl . Calibration was made against samples of the original spray solution

Area of the sprayed leaves was measured using the Delta-T Scan computer package (Delta-T Devices Ltd, Burwell, Cambridge, UK). The amount of spray drift intercepted by the plants was then calculated as $\mu\text{l deposit mm}^{-2} \text{ pass}^{-1}$ made by the tractor mounted sprayer.

3.2.1 Statistical Analyses

Deposits $\text{mm}^{-2} \text{ pass}^{-1}$ were $\log x + 1$ transformed to assume a normal distribution. Power-law regression analysis was used to explore the relationship between distance from sprayer and spray drift interception by all plants (P. Miller, Silsoe Research Institute, pers. comm.). However, for individual species it was more appropriate to use contrast analysis as an *a priori* test in ANOVA (Sokal & Rohlf, 1995) to detect any variance in the amount of drift interception in the buffer strips rather than use regression analysis, since the number of replicates was reduced to five for each species at three distances (P. Miller, Silsoe Research Institute, pers. comm.).

Linear regression was used to explore the relationship between height of plant and drift interception and between leaf area and drift interception. Two-way ANOVA with leaf texture and shape as factors was used to test for variance in drift interception between hairy and non-hairy leaved plants with texture as main effect and between oval, pinnate, long and divided leaves with shape as main effect.

3.3 Results

A one-way ANOVA with day of experiment as main effect indicated that drift deposition on all plants did not significantly differ between the days and therefore the five experiments were assumed to be true replicates ($F_{(4, 235)} = 1.98, P > 0.05$).

3.3.1 Distance from Sprayer

The width of buffer strip, and therefore distance from sprayer, affected the amount of spray drift intercepted by all plants. Power-law regression analysis showed a significant exponential decrease in spray drift interception with increase in distance from sprayer and accounted for 51% of the variation in drift interception by plants ($F_{(1, 238)} = 250.04$, $P < 0.001$; $r^2 = 0.51$) (Figure 3.2).

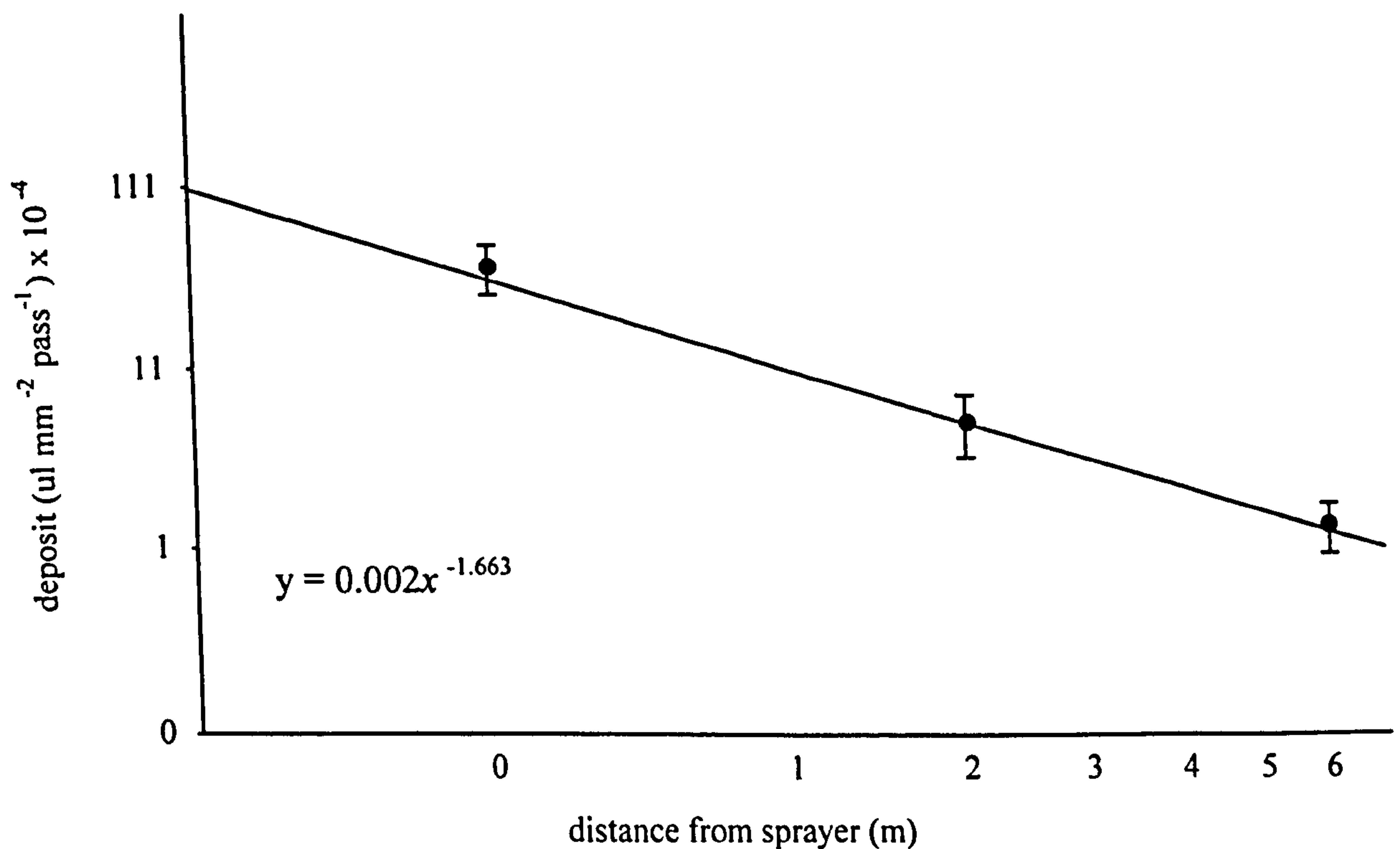


Figure 3.2. Relationship between mean drift deposition on plants ($\mu\text{l mm}^{-2} \text{ pass}^{-1}$) and distance from sprayer. Scales are logarithmic; Error bars are $1.96 \times \text{SE}$.

The amount of spray deposit intercepted by most plant species was shown to vary between the three distances from the sprayer, therefore, contrast analysis was used to identify where these differences lay. The amount of drift intercepted by all of the individual plant species at 6m from the sprayer was not significantly different from amounts intercepted at 2m. Most species,

however, intercepted significantly less drift at 2m and 6m away from the sprayer than in the fully sprayed strip (Table 3.4). *D. glomerata* and *C. vulgare* were the exceptions, where spray drift interception was not significantly reduced 2m away from the sprayer for both species, and also at 6m away from the sprayer for *C. vulgare*.

Table 3.4. Mean, SE and contrast analysis significance values for differences in mean deposit ($\mu\text{l mm}^{-2} \text{ pass}^{-1}$) intercepted in the fully sprayed strip (0m) and at 2m away from the sprayer and, in the fully sprayed strip (0m) and at 6m away from the sprayer.

	Mean Deposit ($\times 10^{-4}$) with SE						0m & 2m		0m & 6m	
	0m		2m		6m		$F_{(2, 12)}$	P	$F_{(2, 12)}$	P
<i>R. obtusifolius</i>	116.83	± 31.52	6.84	± 1.39	3.43	± 2.24	18.14	0.001	19.28	0.001
<i>A. elatius</i>	133.62	± 35.31	28.61	± 7.34	5.18	± 1.26	12.70	0.004	19.00	0.001
<i>E. repens</i>	11.62	± 3.20	2.33	± 0.62	0.65	± 0.18	12.12	0.005	16.90	0.001
<i>S. media</i>	23.67	± 7.45	2.02	± 0.60	0.74	± 0.22	12.58	0.004	14.11	0.003
<i>F. rubra</i>	100.53	± 31.87	19.40	± 7.20	1.70	± 0.35	9.25	0.010	13.72	0.003
<i>C. holosteoides</i>	8.98	± 3.38	0.35	± 0.09	0.17	± 0.06	9.76	0.009	10.16	0.008
<i>T. maritimum</i>	19.68	± 7.00	2.50	± 0.52	1.35	± 0.36	8.96	0.011	10.20	0.008
<i>S. alba</i>	8.39	± 2.87	1.72	± 0.33	0.58	± 0.20	7.93	0.016	10.87	0.006
<i>C. arvense</i>	17.38	± 6.62	1.34	± 0.56	0.46	± 0.11	8.74	0.012	9.73	0.009
<i>L. perenne</i>	9.91	± 3.62	2.03	± 1.06	0.47	± 0.22	6.52	0.025	9.38	0.010
<i>L. album</i>	10.72	± 4.42	2.63	± 0.72	0.84	± 0.38	4.86	0.048	7.24	0.020
<i>A. stolonifera</i>	13.15	± 6.40	1.15	± 0.27	0.42	± 0.14	5.26	0.041	5.92	0.032
<i>C. nigra</i>	27.99	± 13.02	3.45	± 1.30	1.96	± 1.18	5.23	0.041	5.89	0.032
<i>G. robertianum</i>	64.99	± 32.79	2.37	± 1.08	1.61	± 0.49	5.46	0.038	5.60	0.036
<i>D. glomerata</i>	43.68	± 20.66	10.61	± 5.47	2.50	± 1.22	3.58	0.083	5.55	0.036
<i>C. vulgare</i>	37.20	± 22.29	2.84	± 0.61	1.20	± 0.27	3.56	0.084	3.91	0.071

Significant values at $P < 0.05$ in bold.

3.3.2 Plant Species

There was a significant interaction between amount of deposition on plant species and amount of deposition in width of buffer strip ($F_{(30, 192)} = 3.97$, $P < 0.001$), therefore deposition of drift on plant species was analysed separately for each width of buffer strip. There were significant

inter-specific differences in the amount of spray drift interception in each of the three buffer strips (Table 3.5).

Table 3.5. ANOVA of the amount of spray drift intercepted by different species ($\mu\text{l mm}^{-2} \text{ pass}^{-1}$) in the fully sprayed, 2m wide and 6m wide buffer strips.

Buffer Strip	$F_{(15, 64)}$	P
fully sprayed	4.80	<0.001
2m	6.70	<0.001
6m	2.03	<0.027

Significant values at $P<0.05$ in bold.

The mean amount of spray drift intercepted by the species in each of the buffer strips is illustrated in Figures 3.3 - 3.5, where most species intercepted similar amounts of drift.

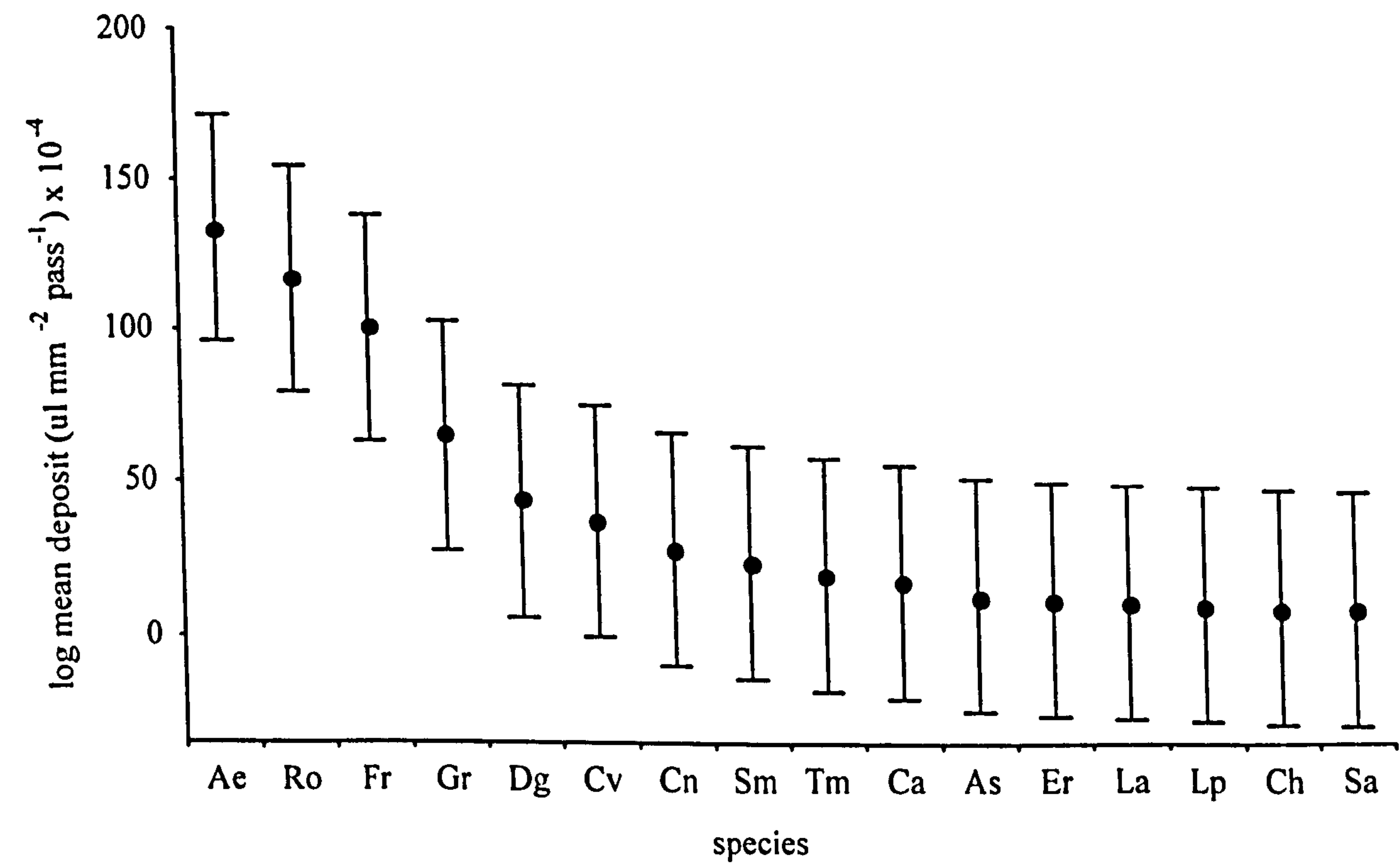


Figure 3.3. Spray deposits ($\mu\text{l mm}^{-2} \text{ pass}^{-1}$) on plant species in the fully sprayed strip. Error bars are 1 x SE. Refer to Table 3.1 for key to abbreviations.

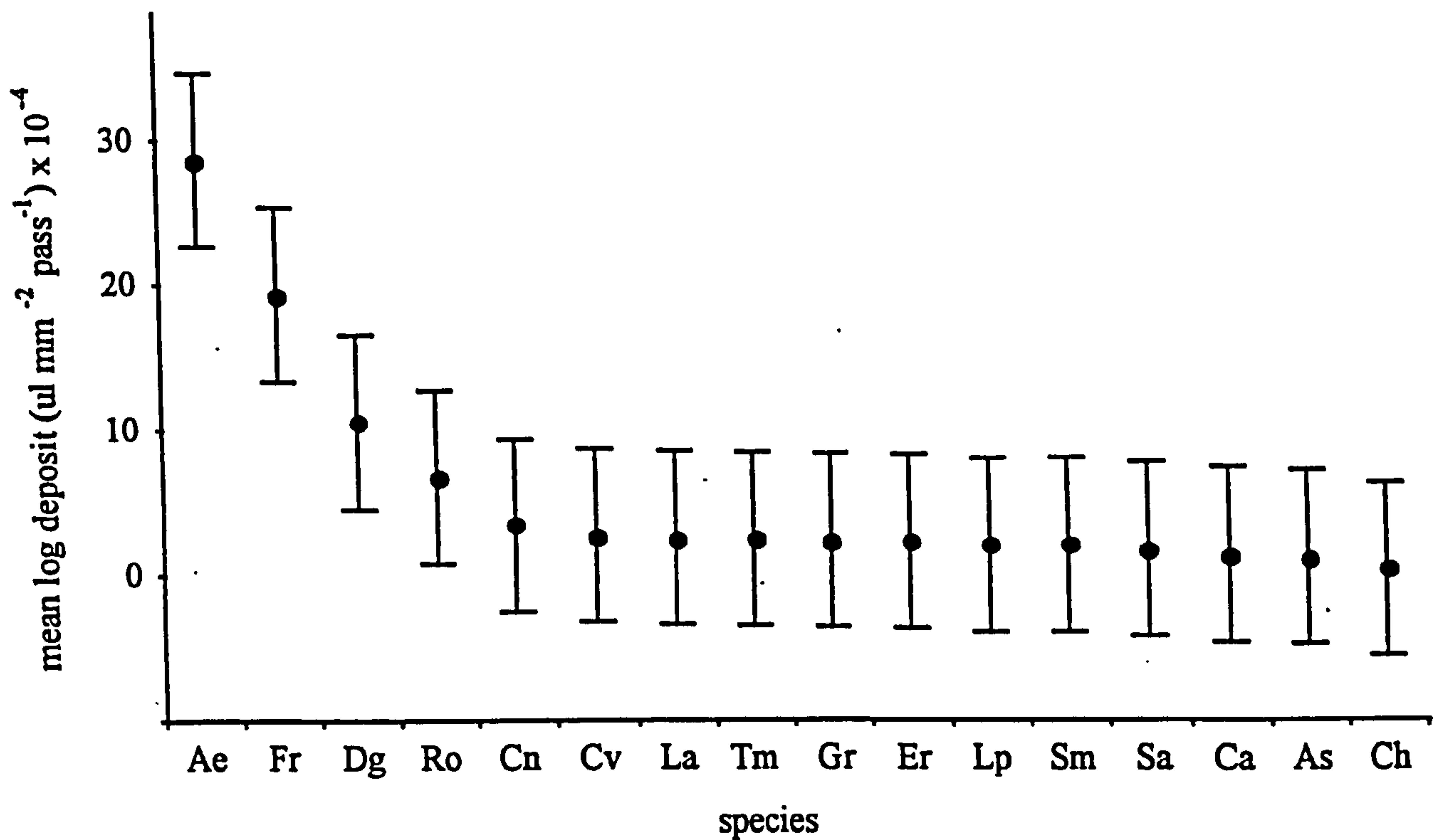


Figure 3.4. Spray deposits ($\mu\text{l mm}^{-2} \text{ pass}^{-1}$) on plant species in the 2m wide buffer strip. Error bars are 1 x SE. Refer to Table 3.1 for key to abbreviations.

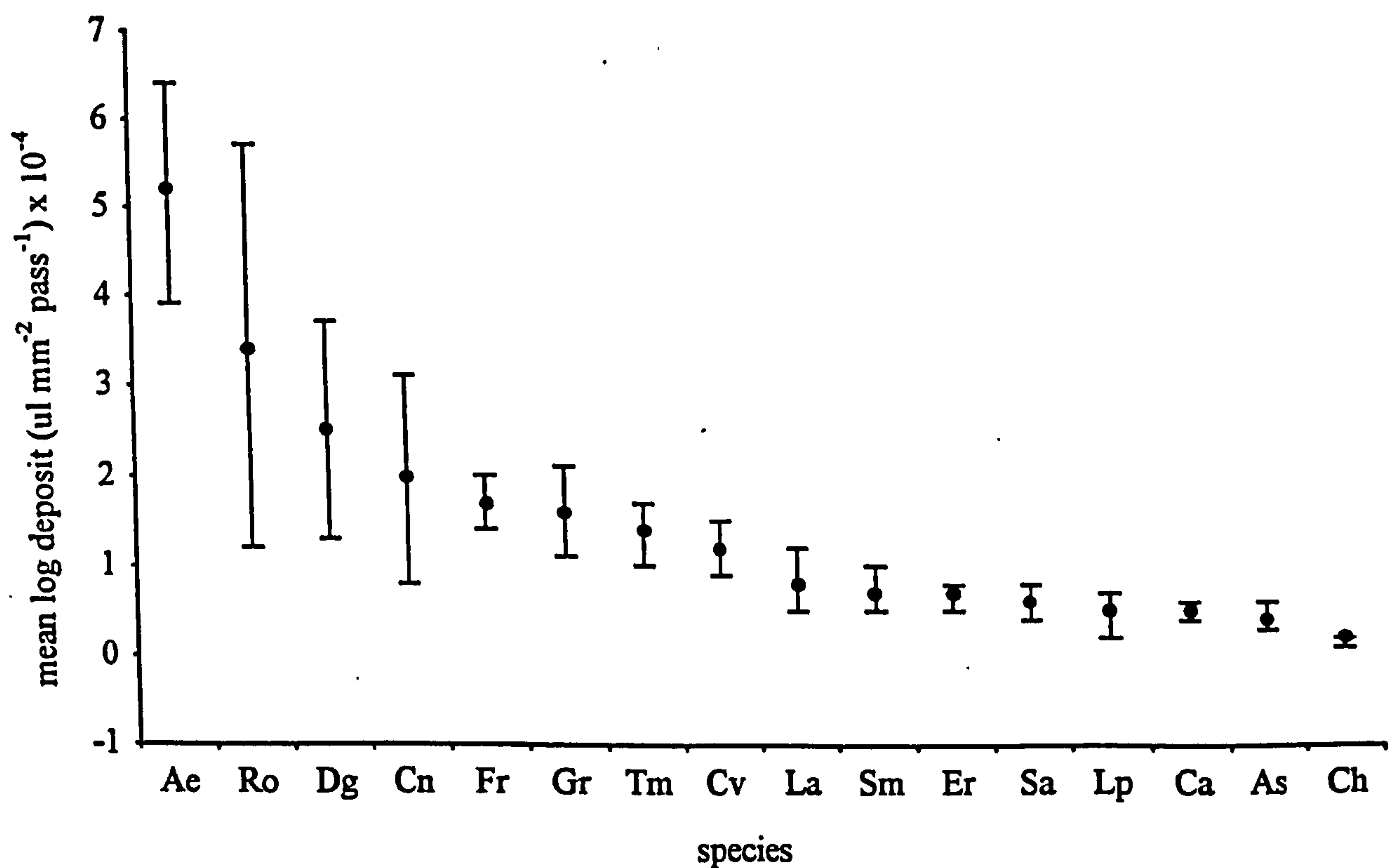


Figure 3.5. Spray deposits ($\mu\text{l mm}^{-2} \text{ pass}^{-1}$) on plant species in the 6m wide buffer strip. Error bars are 1 x SE. Refer to Table 3.1 for key to abbreviations.

Contrast analysis showed that *A. elatius* and *R. obtusifolius*; *A. elatius* and *F. rubra*; and, *A. elatius* intercepted significantly more drift than some other species in the fully sprayed strip, 2m wide and 6m wide buffer strips respectively (Table 3.6). There were no significant inter-specific differences in drift interception between the remaining species (Table 3.6).

Table 3.6. Contrast analysis significance values for amount of spray drift intercepted by species that intercepted significantly different amounts of drift to other species in the same buffer strip.

	0m		2m		6m
	<i>A. elatius</i>	<i>R. obtusifolius</i>	<i>A. elatius</i>	<i>F. rubra</i>	<i>A. elatius</i>
<i>A. stolonifera</i>	0.003	0.029	0.001	0.007	0.011
<i>A. elatius</i>	-	1.000	-	1.000	-
<i>C. nigra</i>	0.022	0.170	0.001	0.045	0.760
<i>C. holosteoides</i>	0.002	0.017	0.001	0.004	0.005
<i>C. arvense</i>	0.006	0.048	0.001	0.008	0.013
<i>C. vulgare</i>	0.070	0.475	0.001	0.028	0.106
<i>D. glomerata</i>	0.150	0.938	0.009	1.000	1.000
<i>E. repens</i>	0.003	0.024	0.001	0.019	0.022
<i>F. rubra</i>	1.000	1.00	1.000	-	0.400
<i>G. robertianum</i>	1.000	1.00	0.001	0.019	0.315
<i>L. album</i>	0.002	0.021	0.001	0.024	0.039
<i>L. perenne</i>	0.002	0.019	0.001	0.015	0.013
<i>R. obtusifolius</i>	1.000	-	0.001	0.518	1.000
<i>S. alba</i>	0.002	0.016	0.001	0.011	0.018
<i>S. media</i>	0.013	0.103	0.001	0.014	0.029
<i>T. maritimum</i>	0.008	0.064	0.001	0.021	0.161

Significant values at $P < 0.05$ in bold.

3.3.3 Leaf Area

There was a significant positive relationship between leaf area and the amount of spray drift (per mm²) across all buffer strips ($F_{(1, 238)} = 4.15$, $P < 0.05$), but leaf area accounted for only 2% of the variation. When effect of leaf area on spray interception was analysed in each of

the three buffer strips, it was found that larger leaves intercepted significantly more spray drift than smaller leaves in the 2m wide buffer strip only, but this relationship was marginal and accounted for only 5% of the variation in spray drift interception (Table 3.7).

Table 3.7. Regression parameters for effect of leaf area on spray drift interception in each buffer strip.

Buffer Strip	a	b	r ²	P
fully sprayed	2.23	0.001	0.02	0.197
2m	0.74	0.001	0.05	0.046
6m	0.37	0.001	0.01	0.536

Significant values at $P < 0.05$ in bold.

3.3.4 Plant Height

There was a significant positive relationship between the height of plants and amount of spray drift deposits per mm² across all strips ($F_{(1, 238)} = 6.83$, $P < 0.01$) however height of plants accounted for only 3% of the variation. When the effect of plant height on spray drift interception was examined for each of the three buffer strips, it was found that taller plants intercepted significantly more spray drift than shorter plants in the 2m and 6m wide strips, but not in the fully sprayed strip (Table 3.8).

Table 3.8. Regression parameters for the effect of plant height on the interception of spray drift in each buffer strip.

Buffer Strip	a	b	r ²	P
fully sprayed	1.70	0.194	0.02	0.210
2m	-0.65	0.449	0.32	0.001
6m	-0.39	0.232	0.25	0.001

Significant values at $P < 0.05$ in bold.

3.3.5 Leaf Texture

There were no significant differences in amount of spray drift deposits intercepted by hairy and non-hairy leaves in all the strips combined ($F_{(1, 232)} = 0.18, P > 0.05$) or in each of the buffer strips (Table 3.9).

Table 3.9. ANOVA results for differences in drift deposits ($\mu\text{l mm}^{-2} \text{ pass}^{-1}$) on hairy and non-hairy leaved plants in the fully sprayed, 2m wide and 6m wide buffer strips.

Buffer Strip	Mean Deposit x 10 ⁻⁴				F _(1, 78)	P
	Hairy		Non-Hairy			
fully sprayed	26.71	± 10.74	46.71	± 9.46	2.00	0.162
2m	2.57	± 1.77	5.50	± 1.56	1.57	0.241
6m	1.10	± 0.40	1.50	± 0.36	0.59	0.447

3.3.6 Leaf Shape

The crude measure of leaf shape of the plant species had no effect on the amount of spray drift interception either across the three buffer strips combined ($F_{(3, 236)} = 0.91, P > 0.05$) or in each of the three buffer strips (Table 3.10).

Table 3.10. ANOVA results for differences in drift deposits ($\mu\text{l mm}^2 \text{ pass}^{-1}$) on differently shaped leaves in the fully sprayed, 2m wide and 6m wide buffer strips.

Buffer Strip	Mean Deposit x 10 ⁻⁴								F _(3, 76)	P
	Long	Oval		Divided		Pinnate				
0m	41.50 ± 10.64	35.72 ± 11.16	42.33 ± 17.51	27.29 ± 17.51	0.19	0.902				
2m	8.61 ± 1.76	3.00 ± 1.84	2.43 ± 2.89	2.09 ± 2.89	2.32	0.083				
6m	1.69 ± 0.40	1.19 ± 0.42	1.48 ± 0.66	0.83 ± 0.66	0.50	0.686				

3.4 Discussion

Fluorescein is the most widely used fluorescent tracer, however, it is important to consider its limitation of photo-instability. When comparing spray tracers, Cross *et al.* (1997) found that fluorescent dyes were photo-unstable, and fluorescein in particular has been shown to degrade in strong northern latitude sunlight to 87% after 35 minutes (Cooke & Hislop, 1993). Therefore, the amount of time that leaves receiving fluorescein spray drift and exposed to light in this experiment was kept to less than 30 minutes to ensure maximum recovery of the tracer.

Increasing the distance between the sprayer and non-target plants significantly reduced the amount of spray drift interception. Spray drift intercepted by plants was exponentially reduced with increasing distance from the sprayer, thus, small distances from the sprayer resulted in great reductions in drift. This pattern of non-linear reduction in spray drift deposition with increasing distance from the sprayer has also been recorded in arable farm crops (Davis *et al.*, 1993c; Ganzelmeier *et al.*, 1995; Longley & Sotherton, 1997b). In this experiment, when spray drift interception by leaves (per mm²) in the fully sprayed strip was compared with that in the 2m and 6m wide strips, drift interception was reduced by 85% and 95% respectively. Cuthbertson & Jepson (1988) also recorded similar significant reductions in spray drift intercepted by artificial targets (plastic drinking straws) placed in a 6m wide buffer strip compared with a fully sprayed strip, where reductions of up to 75% were recorded.

At a species level, the 2m and 6m wide buffer strips significantly reduced the interception of spray drift for most species, but there was no significant difference in amounts of drift intercepted by each species between these two widths of buffer strip. Furthermore, despite spray drift interception by all plants being significantly reduced by the 2m and 6m wide buffer

strips when compared with that in the fully sprayed strip, interception by some individual species *per se* was not significantly reduced in these strips. *D. glomerata* did not receive significantly less spray drift in the 2m wide buffer strips than in the fully sprayed strip, while spray drift intercepted by *C. vulgare* was not significantly reduced by either the 2m or the 6m wide buffer strip. Thus, for *D. glomerata*, buffer strips need to be 6m wide to effectively reduce exposure to spray drift, while for *C. vulgare*, buffer strips ought to be greater than 6m wide. For the other species, however, a 2m wide buffer strip was effective in reducing significant exposure to spray drift.

Variation in spray drift interception amongst plant species differed between each of the buffer strips, where it was at its greatest with the 2m wide buffer strip and at its least with the 6m wide strip. This suggests that more species intercepted significantly different amounts of drift to each other with the 2m wide buffer strip than with the 6m wide buffer strip, where drift interception was more evenly distributed between the species. Thus, some species may be more prone to intercepting spray drift than others at short distances from the sprayer. For example, *A. elatius* intercepted more drift than many other species in each of the three strips, while *R. obtusifolius* and *F. rubra* did in the 0m and 2m wide strips respectively. All other species intercepted similar amounts of spray drift in each of the buffer strips. This experiment illustrates that while a 2m wide buffer strip is extremely effective in reducing spray drift into non-target areas, even at 6m downwind from the sprayer, measurable amounts of spray drift can be deposited on plants. When examining appropriate distances from the sprayer to protect plant species from various effects of herbicides, Marrs *et al.* (1992b) found that buffer zones of between 6m and 10m would be adequate to protect most established perennials from severe damage and growth reduction, while for the most sensitive establishing seedlings, this distance would need to be increased to 20m. However, in order that plants avoid lethal effects of

herbicides, 6m was deemed the maximum safe distance from the sprayer, while 2m was adequate as a minimum safe distance (Marrs *et al.*, 1989a).

In exploring possible reasons for interspecific differences in spray drift interception, the effects of leaf area, plant height, leaf texture and leaf shape on drift capture were considered. The size of leaves only accounted for a very small amount of the variance in spray drift interception over the combined distances from the sprayer and in the 2m wide buffer strip only. Therefore the size of leaf was not very important in determining the potential amount of spray drift interception by the plants. The reasons behind this may be linked to other leaf characteristics that were not measured, and the angle of leaf to the spray drift droplets.

Height of plants accounted for very little variance in spray drift interception over the combined distances from the sprayer and in the fully sprayed strip, but in the 2m wide and 6m wide buffer strips it accounted for 32% and 25% respectively of the variance in drift interception.

These results support those of Cuthbertson & Jepson (1988) who predicted that taller plants 6m away from the sprayer would be exposed to higher maximum drift levels than shorter plants, whereas there was no such prediction for plants adjacent to a fully sprayed strip. Miller & Lane (1999) also found that taller plants intercepted greater amounts of drift downwind from the sprayer. The changes in the influence of plant height on spray drift interception at different distances from the sprayer are probably due to the nature of the cloud of spray drift.

In the fully sprayed strip the cloud of spray is more dense and therefore more likely to completely envelop the plants. At distances further away from the sprayer, the spray cloud becomes more diffuse and only likely to come into contact with the taller plants, because shorter plants receive some protection from the neighbouring vegetation. Since smaller spray droplets are more prone to lateral movement and hence drift than larger ones, those plants that

have a greater vertical than horizontal area are more efficient filters of spray drift (Elliott & Wilson, 1983). Taller plants have a greater vertical area and are therefore more exposed to spray drift.

While taller plants (and associated fauna) in buffer strips may be more susceptible to the effects of pesticide drift than shorter plants, they also have the effect of sheltering vegetation downwind of the sprayer, which may be useful for the protection of vulnerable flora and fauna communities. Longley & Sotherton (1997b) noted that increased drift deposits in field boundaries under an autumn spray regime compared with a summer regime could be attributed to the absence of a mature crop and tall margin vegetation that were acting as spray drift interceptors.

Although it was expected that hairy leaves would intercept more spray drift than non-hairy leaves due to their ability to intercept small spray droplets (Davis & Williams, 1993), this did not happen. There were no significant differences in amount of spray drift deposits on hairy and non-hairy leaves either in the three strips combined or in individual strips. Furthermore, the shape of leaf did not affect the amount of spray drift interception at any of the distances from the sprayer, or in the combined three strips. It was thought that entire leaves (oval and long) would intercept more drift droplets since they have a greater contiguous surface area.

In retrospect, it would have been useful to measure the leaf area : distance around leaf edge ratio to give a precise measurement, rather than rely on a crude classification of leaf shape, which may have obscured differences in spray drift interception.

It appears, therefore, that because plants adjacent to a fully sprayed strip are exposed to a dense spray cloud, plant height and leaf characteristics such as leaf texture, shape and size are not

important in determining the degree to which plants intercept spray drift, since all plants are wholly enveloped by the spray cloud. At 2m away from the sprayer, the spray drift cloud becomes less dense and therefore those species which are taller and have larger leaves are more prone to intercepting greater amounts of drifting droplets. Finally, at 6m away from the sprayer, when the spray drift cloud has become more diffuse, with droplets travelling in a lateral direction by air currents higher above ground level, only the taller plants are able to intercept the spray droplets. Therefore, in order to protect non-target taller plant species from spray drift using buffer strips, distances of 6m from the sprayer are ideal, while shorter, smaller leaved species benefit from being 2m from the sprayer. Furthermore, buffer strips of *Dactylis glomerata* sown at the crop edge would serve to intercept spray drift, thus protecting non-target habitat to the rear of the crop edge.

4 DOSE RESPONSE OF FOURTEEN PLANT SPECIES TO GLYPHOSATE

4.1 Introduction

Arable field margins support important plant communities in agro-ecosystems and are exposed to direct (de Snoo, 1994) and misplaced (de Snoo & van der Poll, 1999) agrochemical inputs. The effects of agrochemicals on general field margin flora have been well documented, where fertiliser leads to a species poor community (e.g., Perry *et al.*, 1996; Wilson, 1999) and herbicide encourages an annual weed dominated, species poor, flora (e.g., Smith *et al.*, 1993).

Selective herbicides offer control of particular taxonomic groups (e.g. monocotyledons, dicotyledons) and species, while broad spectrum herbicides control across a range of taxonomic groups and species. The impact of direct and misplaced inputs of broad spectrum herbicides on plant species occurring in grassy field margins may be both widespread, due to the non-selective properties of the active ingredient, and variable, as a result of differences in interspecific susceptibilities to the active ingredient. For example, Breeze *et al.* (1992) found that post-spray plant growth response was similar amongst wild plant species treated with broad spectrum and dicotyledon-controlling herbicides, while Marshall & Birnie (1985) recorded variability in damage sustained by plants treated with herbicides that control annual broadleaved species.

Much work has been done on the impact of herbicide drift on plant species at various distances from the sprayer. Marrs *et al.* (1992b) found that many species typical of different semi-natural communities were affected by herbicide at distances of less than 6m downwind of the sprayer, while establishing seedlings were sensitive at up to 20m away from the sprayer.

Interspecific response to herbicides at distances downwind of the sprayer was also found to vary (Marrs *et al.*, 1992b)

This experiment was designed to determine the dose response of 14 species of plant that commonly occur in grassy arable field margins to the broad spectrum herbicide glyphosate.

The aim was also to identify specific susceptibilities and relate these to leaf shape and texture and to predict likely effects of exposure to glyphosate applications.

4.2 Materials & Methods

4.2.1 Plant Species

Fourteen species of grasses and forbs, which commonly occur in grassy arable field margins and represent a variety of leaf size, shape and texture were selected for this experiment. Leaf area was measured by scanning one average sized leaf (determined by eye) from each plant into the Delta-T Scan computer package (Delta-T Devices Ltd, Burwell, Cambridge, UK). Texture of the species' leaves was classified as either hairy or non-hairy and leaf shape was identified as being long, oval, divided or pinnate. Species and their leaf characteristics are presented in Table 4.1.

Thirty individual plants of each species were raised from native seed using John Innes No 2 compost in 10cm pots in late January 1997. The seedlings were weeded and kept in a frost-free poly-tunnel and watered as required. The plants were fed once two weeks before the experiment with a standard fertiliser (N:P:K 6:6:6) according to the instructions (Phostrogen, Deeside, Flintshire), to supplement the original nutrients in the potting compost. Once the danger of frost damage had passed, the pots were moved outside to a rabbit-proof holding area

until they were sprayed (13.vii.1998).

Four doses of glyphosate (Roundup Biactive, Monsanto, High Wycombe, Buckinghamshire, UK) were applied to the maturing pot-grown plants, prior to flowering, using a precision pot sprayer (custom built for Harper Adams University College by J. Reader) using Lurmark 110° flat fan nozzles (03-F110, Longstanton, Cambridge, UK). The doses used were 1800g, 180g, 18g and 1.8g glyphosate ha⁻¹. Five plants of each species for each treatment were arranged randomly in the pot sprayer and treatments were applied at a field volume rate of 200 l ha⁻¹. Five plants of each species remained unsprayed as a control.

The plants remained under cover for two hours immediately after spraying, to prevent any spray being washed off by rainfall, before being placed in a poly-tunnel. After 48 hours, the plants were returned to the rabbit-proof holding area for observation until harvesting. Normal watering resumed once plants were in the holding area, as and when appropriate.

Table 4.1. Plant species treated with glyphosate to record log-dose responses.

Latin Binomial	Common Name	Leaf Area	Leaf Shape	Texture
<i>Silene alba</i> (Sa)	White campion	medium	oval	hairy
<i>Cerastium holosteoides</i> (Ch)	Common mouse-ear	small	oval	hairy
<i>Geranium robertianum</i> (Gr)	Herb-Robert	medium	divided	hairy
<i>Rumex obtusifolius</i> (Ro)	Broad-leaved dock	large	oval	non-hairy
<i>Tripleurospermum maritimum</i> (Tm)	Scentless mayweed	small	divided	non-hairy
<i>Cirsium vulgare</i> (Cv)	Spear thistle	medium	pinnate	hairy
<i>Cirsium arvense</i> (Ca)	Creeping thistle	large	pinnate	non-hairy
<i>Centaurea nigra</i> (Cn)	Black knapweed	medium	long	hairy
<i>Elymus repens</i> (Er)	Couch grass	large	long	non-hairy
<i>Festuca rubra</i> (Fr)	Red fescue	small	long	non-hairy
<i>Lolium perenne</i> (Lp)	Perennial rye-grass	small	long	non-hairy
<i>Dactylis glomerata</i> (Dg)	Cocks foot	medium	long	non-hairy
<i>Arrhenatherum elatius</i> (Ae)	Oat grass	small	long	non-hairy
<i>Agrostis stolonifera</i> (As)	Creeping bent	small	flat	non-hairy

Nomenclature After Stace (1997). Letters in parentheses are abbreviations of species.

4.2.2 Monitoring Effects of Glyphosate

Effects of glyphosate on the plants were recorded using visual damage assessments and dry weight analysis of post-spray biomass. Visible damage to the plants was recorded 10 days after spraying and weekly thereafter, using a 5-point scale from zero (0) which represented no damage to four (4) representing death (Table 4.2).

Table 4.2. Plant health scale.

Score	Damage Criterion
0	no damage
1	yellow tips of leaves
2	yellowed leaves
3	yellowed and wilted leaves
4	dead

Dry weight of the plants was used as a measure of active growth by the plants after spray application. Plants were cut at base level 6 weeks after treatment and the vegetative material was oven-dried at 80°C for 24 hours and then weighed to the nearest 0.001g.

4.2.3 Statistical Analyses

Relationships between mean damage score and rate of glyphosate were explored using Spearman rank correlation and differences in mean damage score between treatments were analysed by Kruskal-Wallis non-parametric analysis of variance (Sokal & Rohlf, 1995).

The response of dry weight of i) all plants and, ii) individual plant species to doses of glyphosate was described non-linear regression using a Genstat program (Release 4.1, Lawes Agricultural Trust, Institute of Arable Crop Research, Rothamsted, UK, 1997) written by P. Brain (IACR, Long Ashton Research Station, University of Bristol, UK). The standard dose-response curve (e.g. Brain *et al.*, 1999) was used to relate weed biomass and herbicide dose (Streibig, 1980) and fitted in the form:

$$y = \frac{w_0}{1 + \left(\frac{e^{dose}}{e^{ED_{50}}} \right)^B}$$

using least squares, where w_0 is the unsprayed weed biomass; ED_{50} is $\log(\text{dose})$ which reduces weed biomass by 50%; and, B is the response rate or steepness of the curve. The ED_{50} was estimated from the data, as the ED_{50} and B are non-linear regression coefficients (Dr Phillip Brain pers. comm.). Differences between mean dry weight between the treatments were analysed using contrast analysis in ANOVA (Sokal & Rohlf, 1995).

Since leaf weight is inherently variable between plant species, the proportional growth of species after treatment compared with untreated specimens was calculated as a ratio to estimate growth response to the glyphosate by the species. Proportional growth was calculated thus:

$$\frac{\text{dryweight of glyphosate treated plant}}{\text{dry weight of untreated plant}}$$

Differences in mean proportional growth between species were analysed using parametric analysis of variance (Sokal & Rohlf, 1995).

4.3 Results

4.3.1 General Effect of Glyphosate on Plants

4.3.1.1 Damage Scores

The visual damage response of the plants became apparent more than two weeks after the spray application and damage scores at week 3 were maintained at the same level until

harvesting at 6 weeks after spray application. Damage was positively related to the dose of glyphosate, however, significant damage (where more than just the tips of leaves were yellowed) was sustained by plants treated with the 1800g glyphosate ha⁻¹ treatment only. Rates of glyphosate equal to and less than 180g ha⁻¹ did not significantly damage the plants, since mean damage scores from weeks 3 to 6 indicated no damage or very slight yellowing of the tips of leaves (Table 4.3).

Table 4.3. Spearman rank correlation coefficients for the relationship between damage scores and rate of glyphosate.

Weeks	Mean Damage Scores					R	P
	control	1.8g	18g	180g	1800g		
2	0	0	0	0	0	-0.01	0.999
3-6	0.01	0.00	0.04	0.05	2.90	0.62	0.001

Significant values at $P < 0.05$ in bold.

Since significant damage (where leaves had yellowed) was confined to plants treated with the 1800g ha⁻¹ glyphosate, the effects of leaf area, texture and shape on damage score were examined in that treatment only. There was no relationship between leaf area and mean damage score in the 1800g treatment at any week after the glyphosate application, thus plants with larger leaves were not more damaged than smaller leaves (Table 4.4).

Table 4.4. Spearman rank correlation coefficients for relationship between leaf area and mean damage scores in all plants.

Weeks after spray application	Spearman R	P
2	-	-
3	0.09	0.078
4	0.03	0.541
5	0.03	0.557
6	-0.01	0.960

Damage scores did not vary between hairy and non-hairy leaves in the 1800g treatment at any week after spray application. The damage scores did, however, vary between leaf shape, but only in weeks 3, 4 and 5. In each case, plants with pinnate leaves were significantly more damaged than the other shaped leaves and in weeks 3 and 5, divided leaves were significantly less damaged than oval leaves (week 3) and long leaves (week 5) (Table 4.5).

Table 4.5. Significant Kruskal-Wallis values for mean damage scores between oval, long, pinnate and divided leaves in different treatments in the weeks after spray application for 1800g. glyphosate ha⁻¹ treatment.

Week	Mean damage scores with SE				<i>H</i>	<i>P</i>
	long	oval	pinnate	divided		
3	3.0 ± 0.2	2.3 ± 0.5	3.9 ± 0.1	1.5 ± 0.5	16.74	0.001
4	2.8 ± 0.3	2.3 ± 0.5	4.0 ± 0.0	1.9 ± 0.6	11.25	0.011
5	3.1 ± 0.3	3.3 ± 0.2	4.0 ± 0.0	1.9 ± 0.6	11.60	0.009
6	3.0 ± 0.3	3.3 ± 0.2	3.6 ± 0.4	2.1 ± 0.6	4.65	0.199

Significant values at $P < 0.05$ in bold.

4.3.1.2 Dry Weight

Increasing the rate of glyphosate resulted in a decrease in dry weight and therefore growth of plants, where rate of glyphosate accounted for 17.4% of the decrease in weight ($F_{(4, 349)} = 19.36$, $P < 0.001$). The ED₅₀ of glyphosate for the plants was 1568.75g glyphosate ha⁻¹.

The proportional growth of plants did not vary with leaf shape ($F_{(3, 272)} = 1.53$, $P > 0.05$) or between hairy and non-hairy leaved plants ($F_{(1, 272)} = 2.60$, $P > 0.05$). There was no relationship between leaf area and dry weight ($F_{(1, 348)} = 2.59$, $P > 0.05$; $r^2 = 0.01$) indicating that plants with larger leaves were not more adversely affected by glyphosate than smaller leaved plants.

4.3.2 Effect of Glyphosate on Species

4.3.2.1 Damage Scores

Plant species suffered damage that amounted to only slight yellowing of the tips of leaves in the 1.8g, 18g and 180g treatments throughout the course of the experiment, therefore only the significant damage scores from the 1800g treatment are reported here. No plant species showed signs of damage 2 weeks after the spray application, however, from the third week onwards, damage had become apparent for most species. All species except *G. robertianum* and *R. obtusifolius* showed signs of damage, where many were exhibiting yellow and wilted leaves (Table 4.6).

Four weeks after glyphosate application, *R. obtusifolius* was the only species to remain visually undamaged. Whereas at 3 weeks post spray application, damage in *E. repens* was significant (yellowed and wilted leaves), this species appeared to have recovered by the 4th week since visible damage had lessened to slight yellowing of tips of leaves. *C. nigra* had also started to recover by week 4, while the other species continued to respond as in the previous week.

By the fifth week after spray application, *R. obtusifolius* exhibited signs of damage (yellowed and wilted leaves) for the first time, while *E. repens* maintained its recovery, remaining in the same condition as in week 4. All other species continued to respond as in the previous two weeks.

At 6 weeks after glyphosate application, *L. perenne* and *C. vulgare* appeared to be recovering from the effects of the herbicide, since the damage scores had decreased. Nevertheless, both

species suffered from yellow and wilted leaves. *F. rubra* continued to exhibit slight damage.

Table 4.6 gives mean damage scores in the weeks after spray application in the 1800g glyphosate ha⁻¹ treatment only.

Table 4.6. Mean damage scores for plants in the 1800g glyphosate ha⁻¹ treatment in the weeks after spray application.

Species	Weeks After Spray Application				
	2	3	4	5	6
<i>A. stolonifera</i>	0.0	4.0	4.0	4.0	4.0
<i>C. holosteoides</i>	0.0	4.0	4.0	4.0	4.0
<i>C. vulgare</i>	0.0	4.0	4.0	4.0	3.2
<i>C. arvense</i>	0.0	3.8	4.0	4.0	4.0
<i>A. elatius</i>	0.0	3.8	3.8	4.0	4.0
<i>D. glomerata</i>	0.0	3.4	3.8	4.0	4.0
<i>T. maritimum</i>	0.0	3.0	3.6	3.6	4.0
<i>S. alba</i>	0.0	2.8	3.0	3.0	3.0
<i>C. nigra</i>	0.0	2.6	2.2	3.0	3.2
<i>L. perenne</i>	0.0	2.4	3.4	4.0	3.2
<i>F. rubra</i>	0.0	1.6	1.6	1.6	1.8
<i>E. repens</i>	0.0	3.0	0.8	0.8	0.8
<i>G. robertianum</i>	0.0	0.0	0.2	0.2	0.2
<i>R. obtusifolius</i>	0.0	0.0	0.0	3.0	3.0

4.3.2.1. Dry Weight

The only treatment to significantly reduce the dry weight of species compared with the untreated control was the 1800g treatment, although not all species' dry weights were significantly reduced (contrast analysis) (Table 4.7).

Table 4.7 Mean dry weight (g) of species in the control and 1800g glyphosate ha⁻¹ treatments and contrast analysis significance values for means in the control and 1800g glyphosate ha⁻¹ treatments.

Species	Mean Dry Weight (g)		$F_{(1, 20)}$	P
	control	1800g		
<i>A. elatius</i>	3.0	1.6	4.71	0.042
<i>A. stolonifera</i>	4.9	1.7	25.18	0.001
<i>C. arvense</i>	3.6	1.1	15.16	0.001
<i>C. holosteoides</i>	2.7	0.9	23.97	0.001
<i>C. nigra</i>	2.7	1.2	4.19	0.054
<i>C. vulgare</i>	6.1	2.3	9.60	0.006
<i>D. glomerata</i>	12.2	6.4	7.40	0.013
<i>E. repens</i>	3.1	1.9	2.91	0.103
<i>F. rubra</i>	3.5	2.2	2.64	0.120
<i>G. robertianum</i>	6.0	4.5	2.68	0.117
<i>L. perenne</i>	2.3	1.5	4.76	0.041
<i>R. obtusifolius</i>	2.9	1.2	14.41	0.001
<i>S. alba</i>	3.6	1.8	4.03	0.059
<i>T. maritimum</i>	3.5	6.2	41.52	0.001

Significant values at $P < 0.05$ in bold.

Mean proportional weight gain of species that were significantly reduced by 1800g glyphosate were compared with proportional weight gain of untreated plants to determine whether inter-species response to the 1800g treatment was variable. Proportional weight gain did not vary significantly between species in the 1800g a.i treatment ($F_{(8, 36)} = 1.29$, $P > 0.05$) (Figure 4.1).

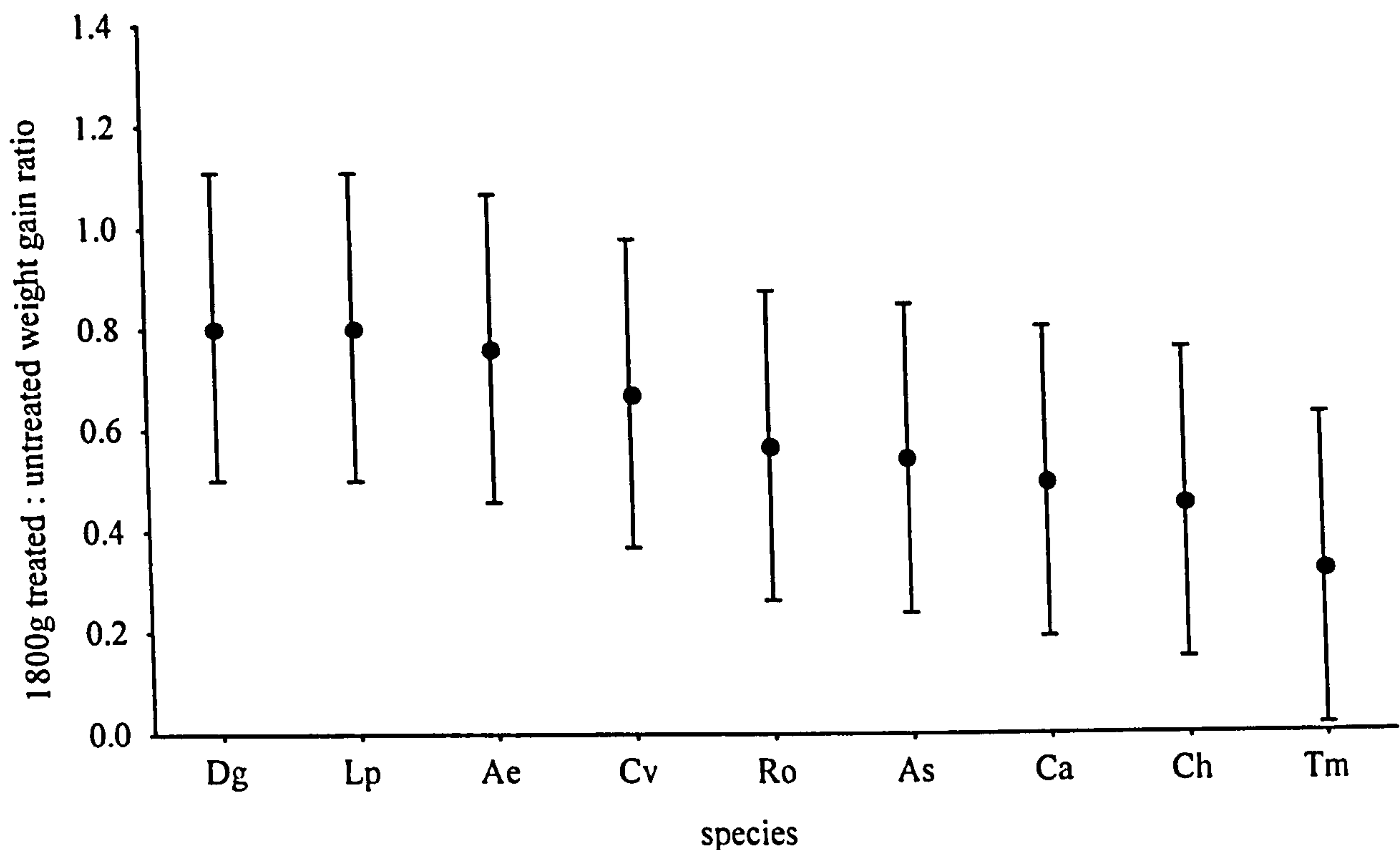


Figure 4.1 1800g glyphosate : untreated weight gain ratio for species whose dry weight was significantly reduced by the 1800g treatment compared with the control. Error bars are 1 x SE.

The dry weight of six of the fourteen species was shown to be related to rate of glyphosate. Figures 4.2 - 4.7 illustrate the dose response relationship between log-dose and log-dry weight of *A. stolonifera*, *C. arvense*, *C. holosteoides*, *C. vulgare*, *D. glomerata*, and *T. maritimum*. Rate of glyphosate accounted for between 22% and 79% of the variation in dry weight. However, dry weights of *A. elatius*, *C. nigra*, *E. repens*, *F. rubra*, *G. robertianum*, *L. perenne*, *R. obtusifolius* and *S. alba* could not be fitted to either a linear regression or the dose response model.

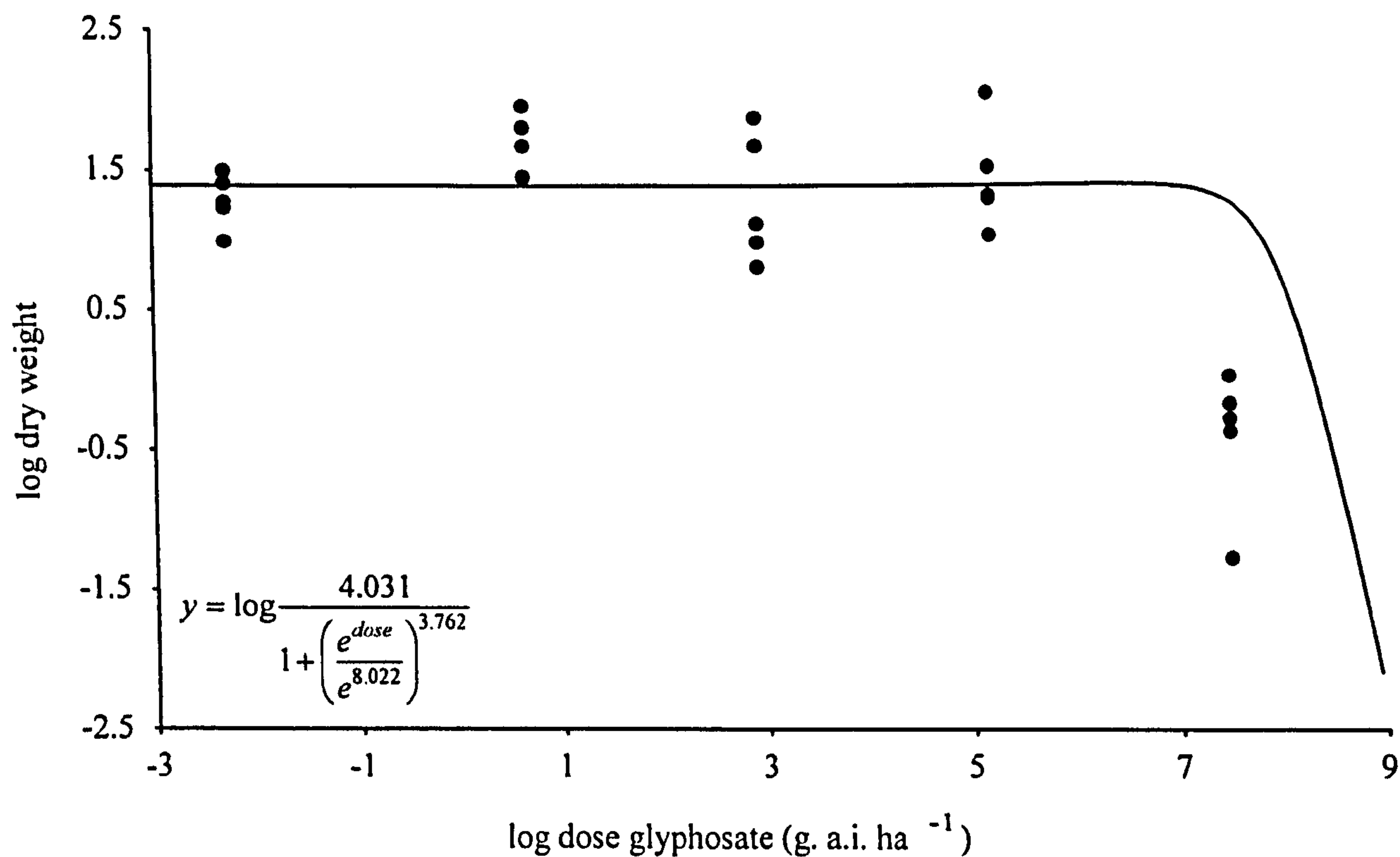


Figure 4.2 Relationship between log dry weight of *Tripleurospermum maritimum* and log dose glyphosate.

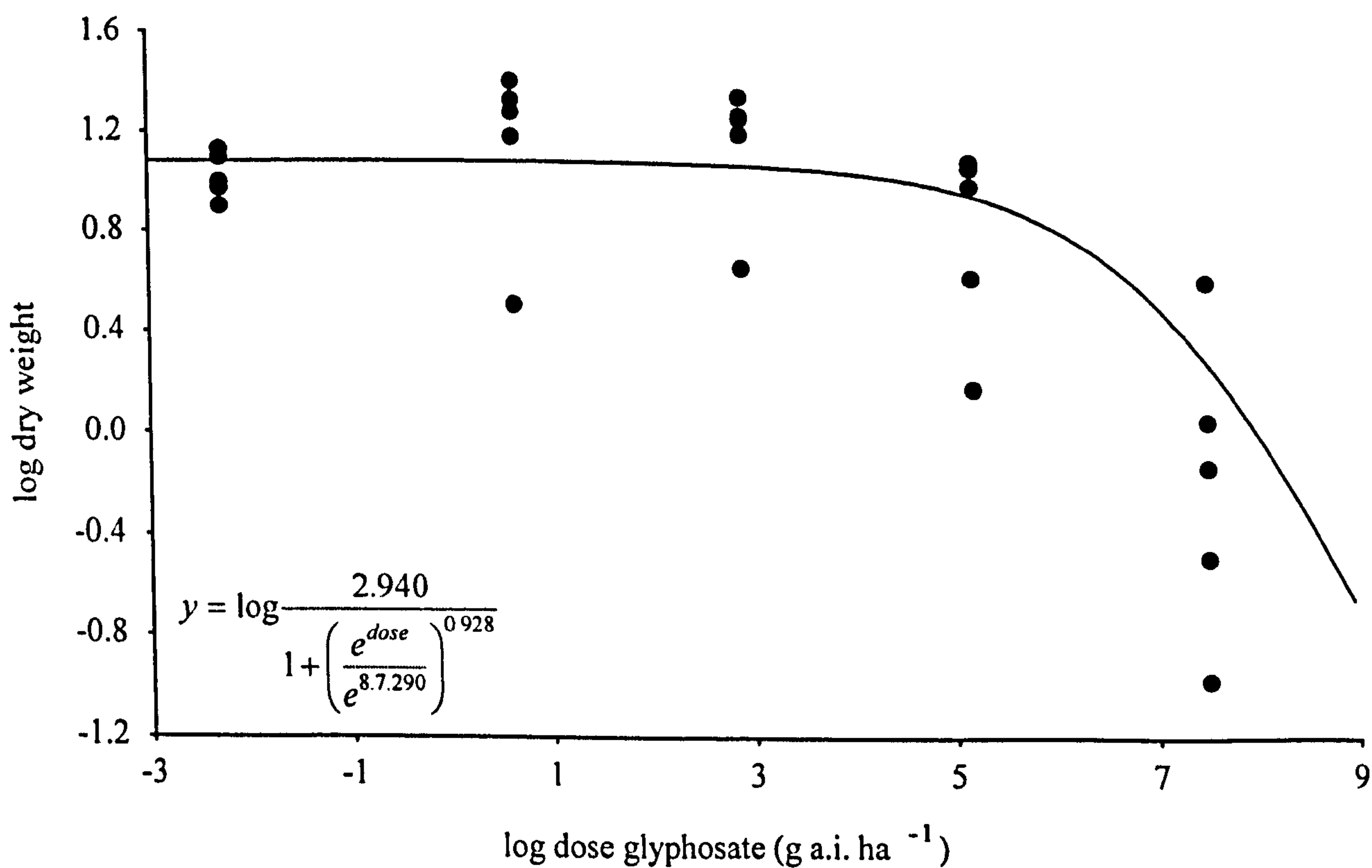


Figure 4.3 Relationship between log dry weight of *Cerastium holosteoides* and log dose glyphosate.

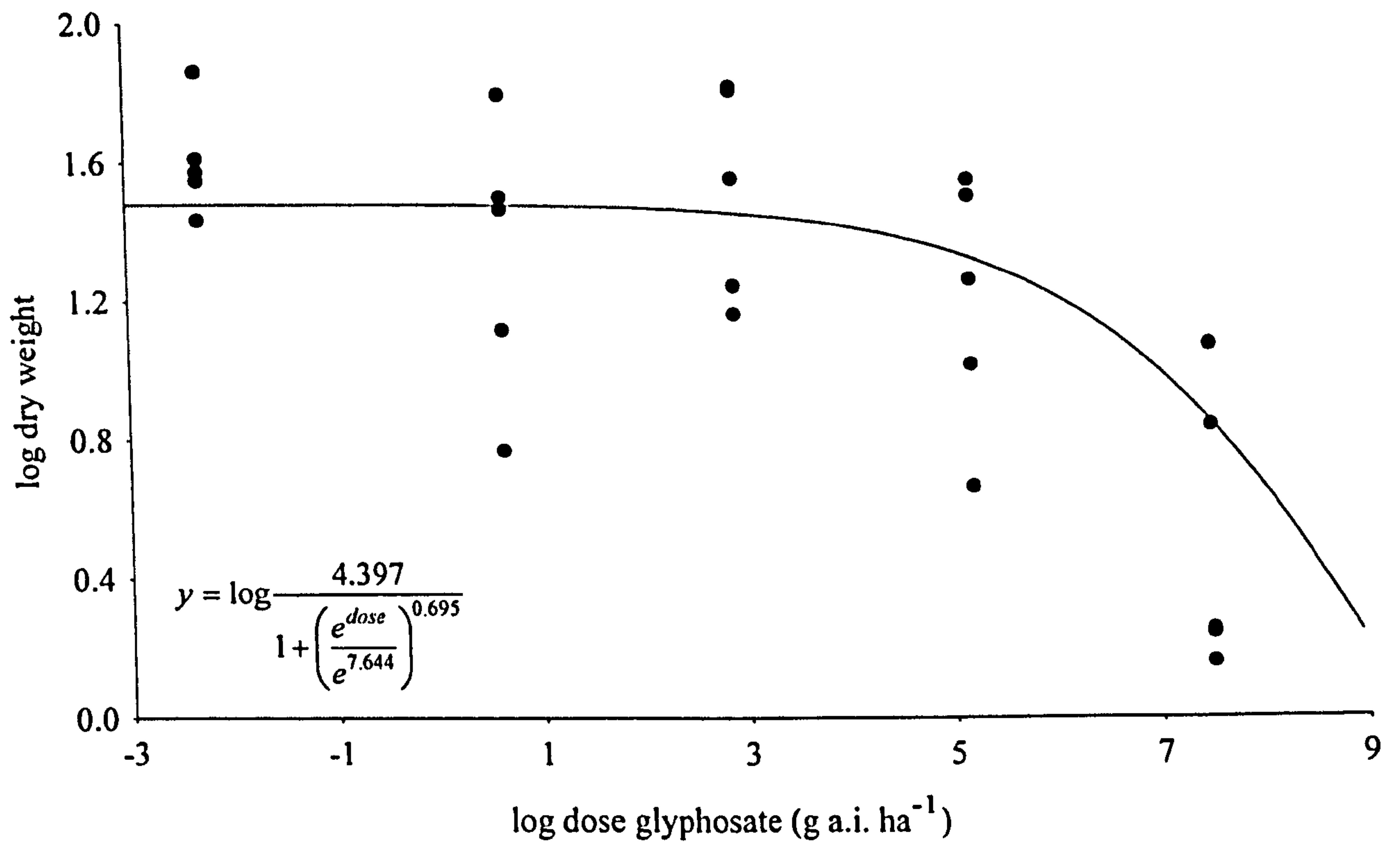


Figure 4.4 Relationship between log dry weight of *Agrostis stolonifera* and log dose glyphosate.

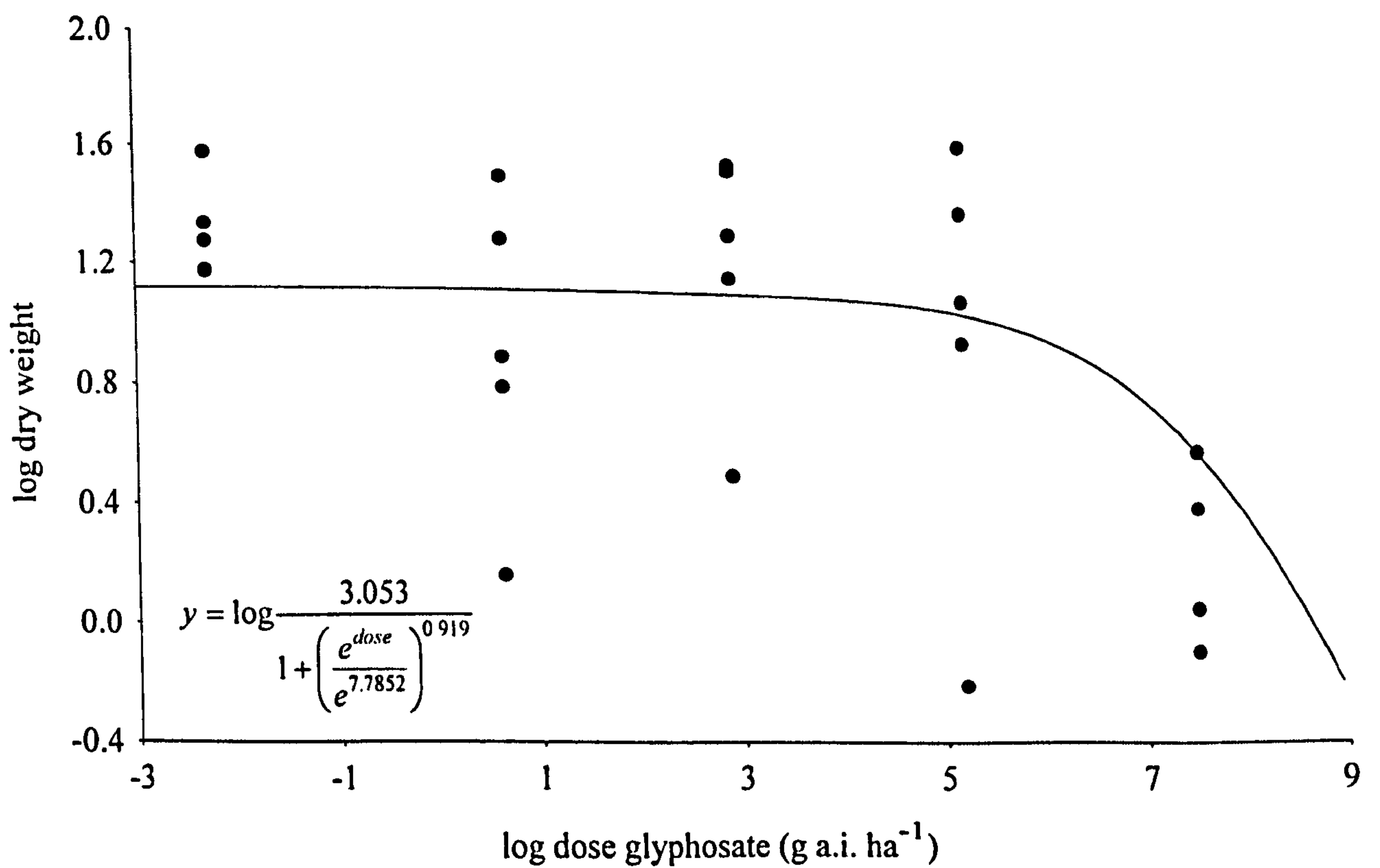


Figure 4.5 Relationship between log dry weight of *Cirsium arvense* and log dose glyphosate.

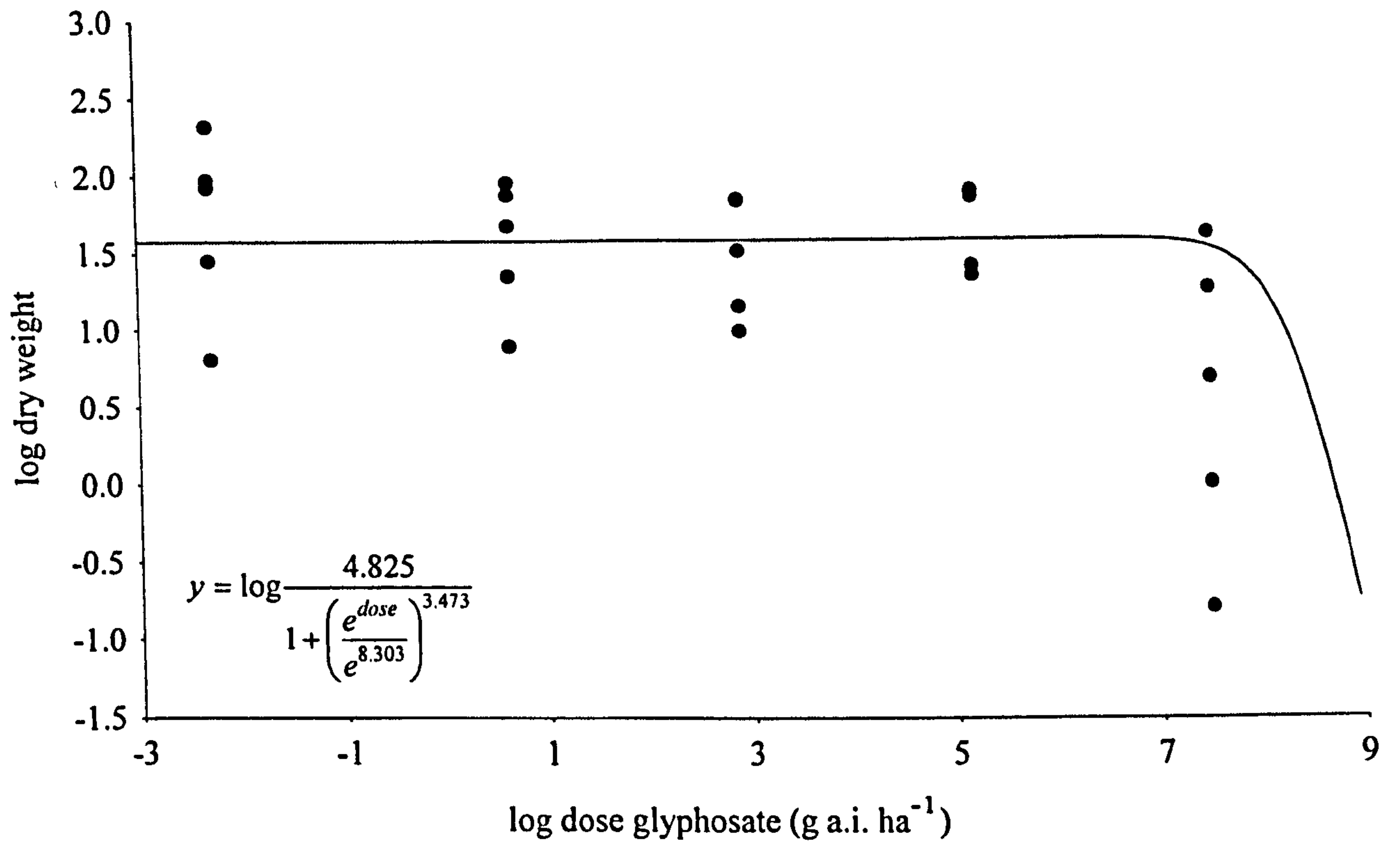


Figure 4.6 Relationship between log dry weight of *Cirsium vulgare* and log dose glyphosate.

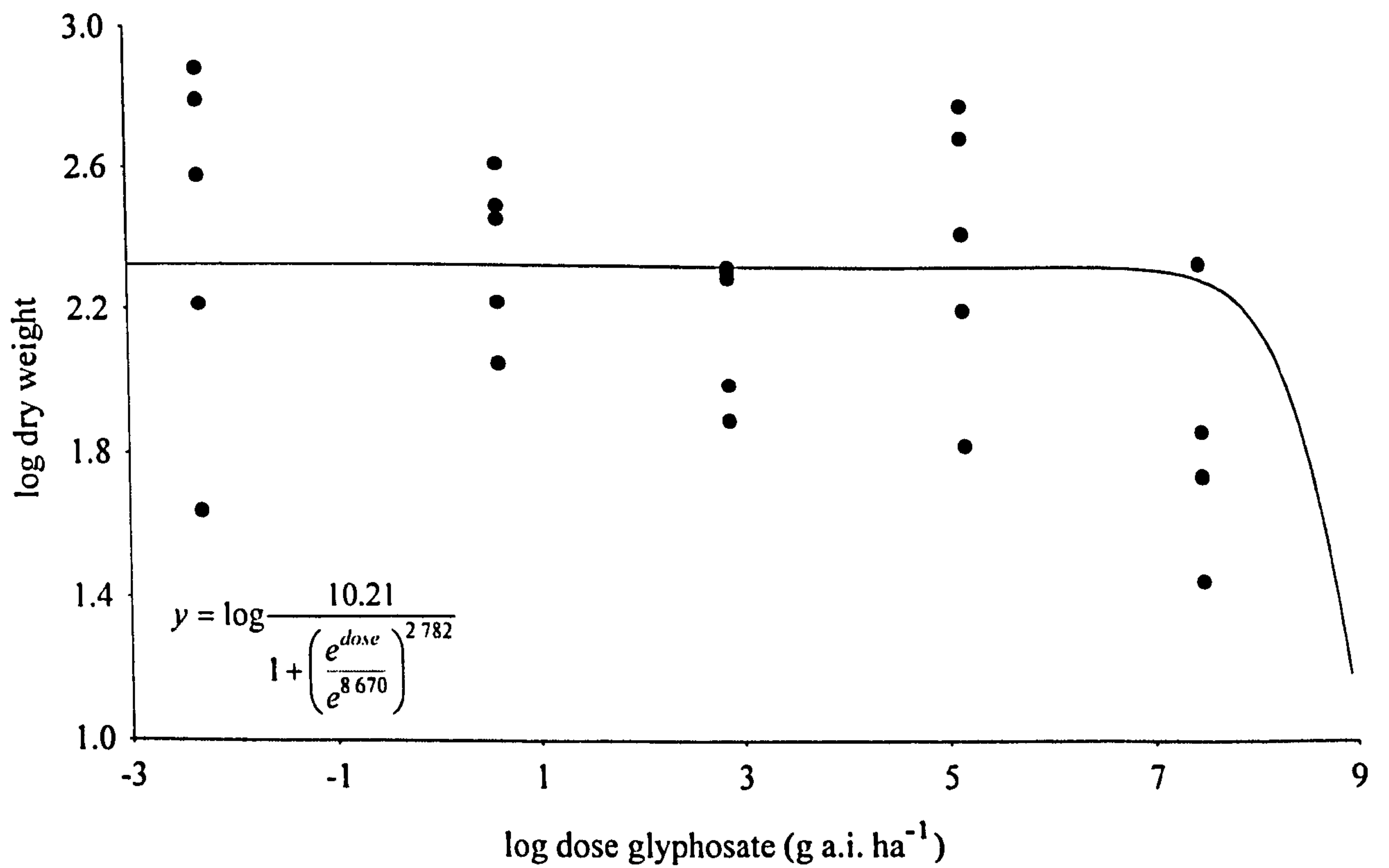


Figure 4.7 Relationship between log dry weight of *Dactylis glomerata* and log dose glyphosate.

ED₅₀s of glyphosate were calculated for the species where a relationship between log dose and log dry weight existed and values ranged from 527.40g to 2098.08g glyphosate ha⁻¹ (Table 4.8).

Table 4.8 ED₅₀ of glyphosate (g ha⁻¹) for species where a relationship between rate of glyphosate and dry weight existed.

Species	ED ₅₀ glyphosate (g ha ⁻¹)
<i>A. stolonifera</i>	751.68
<i>C. arvense</i>	925.20
<i>C. holosteoides</i>	527.40
<i>C. vulgare</i>	1 452.96
<i>D. glomerata</i>	2 098.08
<i>T. maritimum</i>	1 097.64

4.3 Discussion

Visible damage to the plants became apparent from two weeks after the spray application. The mode of action of glyphosate does not allow for an instant effect on plants, because the glyphosate is translocated from the treated shoot growth to roots, rhizomes and stolons (Anon, 1999b), which takes several days to occur. Damage in most plants worsened as the weeks progressed, and peaked at 5 weeks after spray application. *R. obtusifolius* took the greatest amount of time to show signs of damage, whereas *E. repens* was damaged by week 3 and was recovering from week 4. Marshall & Birnie (1985) also found that *R. obtusifolius* was slow to respond to a herbicide (mecoprop) and took 15 weeks to do so. Therefore, the rate at which herbicide affects plants can vary between species.

There was a negative relationship between dry weight and rate of glyphosate and a positive

association between increased damage score and increased rate of glyphosate for all the plants. This indicated that increased rate of glyphosate caused plants to grow less and resulted in more severely damaged plants. The highest rate of glyphosate, which caused significant damage to all plants, was the recommended rate for controlling perennial broadleaved weeds and grasses and annual grasses (Anon, 1999b), whereas the rates equal to and less than 180g ha^{-1} are less than the lowest recommended dose for weed control (Anon, 1999b).

Kirkwood (1987) noted that there can be differential droplet retention between hairy and non-hairy leaves, waxy and non-waxy leaf surfaces and between broadleaved and narrow leaved plants. Differential droplet retention may, therefore, vary the effectiveness of the herbicide. However in this experiment, neither leaf size nor texture were found to influence reduction in plant growth or degree of damage sustained by the plants, but damage score (not dry weight) varied between leaf shape where pinnate leaves were more damaged than the other shaped leaves. Thus, although pinnate leaves were more visibly damaged, this was not reflected in post spray application plant growth. In this experiment, only 2 species had pinnate leaves, and they were con-generics. In order to verify increased susceptibility of pinnate shaped leaves to damage from glyphosate, it is recommended that many more species from different genera, representing this shape of leaf be tested.

There was a general trend for species to become more damaged and grow less when treated with the highest rate of glyphosate, however, the damage and plant growth responses of individual plant species to glyphosate varied between species. Not all species were significantly damaged or grew less when treated with glyphosate, even when treated at the highest rate. *F. rubra* and *G. robertianum*, for example, did not sustain damage greater than slight yellowing of leaves. Furthermore, growth of *C. nigra*, *E. repens*, *F. rubra*, *G.*

robertianum and *S. alba* was neither significantly reduced by the highest rate of glyphosate, nor related to the rate of glyphosate. Therefore, despite *C. nigra*, *E. repens* and *S. alba* showing signs of damage, growth was not significantly affected by glyphosate at the rates recommended for control of such perennial species. *F. rubra* is a difficult grass to control and requires rates of glyphosate greater than 2160 ha⁻¹ (Anon, 1999b), therefore this result is to have been expected. A similar response by native plant species was observed by Marrs *et al.* (1991) where pot-grown plants bore signs of damage, but showed no significant reduction in growth. Marrs *et al.* (1992b) also found the yield of many species treated with a relatively high rate of glyphosate (2 200g ha⁻¹) was not significantly different from plants further downwind of the sprayer which received reduced doses of glyphosate.

The interpretation of the results for *E. repens* must take into account that recovery was well under way by the time the plant was harvested. Therefore, if *E. repens* had been harvested earlier, it is possible that post-spray plant growth may have been reduced by the highest rate of glyphosate. *F. rubra* and *G. robertianum* were, therefore, least sensitive to rates of glyphosate, while *C. nigra*, *E. repens* and *S. alba* were moderately affected. *R. obtusifolius* and *T. maritimum* sustained moderate damage, but post-spray growth was significantly reduced by the highest rate of glyphosate. Therefore for these species, visible damage was a weak indicator of impact on plant growth.

The plants in this experiment were pot-grown and the lack of significant effect of glyphosate, even when treated with the highest rate, may be due to a lack of competition between plants (e.g. Marrs *et al.*, 1991). Plants grown in glasshouses are known to have thinner cuticles and therefore are more prone to herbicide uptake and hence, herbicidal effects (Garrod, 1989). However, the plants were placed outside to increase exposure to ultra-violet light that

promotes cuticle growth, and the results suggest that rather than being thin, cuticles may have been thick, since enhanced response to herbicide was not observed. Furthermore, it is possible that the plants may have not been growing vigorously, despite having been fertilised to compensate for depleted compost nutrient status. Plants must be actively growing in order that glyphosate is translocated efficiently (Anon, 1999b).

All the other species were highly sensitive to glyphosate, both in terms of post-spray growth and damage sustained. Of the species whose weight was significantly reduced by the highest rate, plant growth in all species except *A. elatius*, *L. perenne* and *R. obtusifolius* was related to rate of glyphosate. Therefore, reduction in post spray growth of *A. stolonifera*, *C. arvense*, *C. holosteoides*, *C. vulgare*, *D. glomerata* and *T. maritimum* was related to increasing rate of glyphosate, and ED₅₀s for these species ranged from 752g for *A. stolonifera*, to 2098g for *D. glomerata*. Similar ED₅₀ values have been calculated for some other wild plant species and these ranged from 260g to 1 860g glyphosate ha⁻¹ (Breeze *et al.*, 1992) where the rates of glyphosate fall within the range of doses of between 360g and 2 160g ha⁻¹ recommended for weed control (Anon, 1999b).

Thus, according to these results exposure to glyphosate at rates less than 180g ha⁻¹ would neither significantly affect plant growth nor visible plant health of the species tested here. The sub-lethal effects of rates of glyphosate on the species tested were not assessed so implications for flowering and seed suppression cannot be estimated, although it has been suggested that visibly damaged plants may survive herbicide applications to produce viable seed (Marshall & Birnie, 1985). Exposure to rates greater than 1800g glyphosate ha⁻¹ would, however, significantly reduce the growth of *A. elatius*, *A. stolonifera*, *C. holosteoides*, *C. arvense*, *C. vulgare*, *D. glomerata*, *L. perenne*, *R. obtusifolius*, *T. maritimum*. Increasing applications of

glyphosate would increasingly reduce the plant growth of *A. stolonifera*, *C. arvense*, *C. holosteoides*, *C. vulgare*, *D. glomerata* and *T. maritimum* and applications greater than 527g would reduce their growth by at least 50%.

Since the results of this experiment suggested that many species were not severely affected by high rates of glyphosate that are intended to kill most species (Anon, 1999b), possibly due to the reasons outlined above, the experimental design should be amended. It is suggested that plants are grown in microcosms (see Marrs *et al.*, 1992b) or *in situ* (see Pywell *et al.*, 1996) so that plants are exposed to competition and, spray applications should occur earlier in the season to ensure that plants are actively growing to ensure improved herbicide uptake.

Nevertheless, the results from this experiment suggest that some species may be more sensitive than others to exposure to different rates of glyphosate, which may occur as a direct spray, or as spray drift. To summarise these data, the sensitivities of the plant species tested here to glyphosate applied at the highest rate (1800g ha⁻¹), which is used in set-aside, can be crudely classified into low, moderate and high, based on post-spray plant growth and damage sustained (Table 4.9).

Table 4.9. Sensitivity of plant species to 1800g glyphosate ha⁻¹ based on post-spray plant growth and damage sustained.

Sensitivity	Post-Spray Plant Growth	Damage Sustained
Low	<i>F. rubra</i>	<i>F. rubra</i>
	<i>G. robertianum</i>	<i>G. robertianum</i>
Moderate	<i>C. nigra</i>	<i>C. nigra</i>
	<i>E. repens</i>	<i>E. repens</i>
		<i>R. obtusifolius</i>
	<i>S. alba</i>	<i>S. alba</i>
		<i>T. maritimum</i>
High	<i>A. elatius</i>	<i>A. elatius</i>
	<i>A. stolonifera</i>	<i>A. stolonifera</i>
	<i>C. holosteoides</i>	<i>C. holosteoides</i>
	<i>C. arvense</i>	<i>C. arvense</i>
	<i>C. vulgare</i>	<i>C. vulgare</i>
	<i>D. glomerata</i>	<i>D. glomerata</i>
	<i>L. perenne</i>	<i>L. perenne</i>
	<i>R. obtusifolius</i>	
	<i>T. maritimum</i>	

5 EFFECT ON *LEPTOPTERNA DOLABRATA* (HETEROPTERA: MIRIDAE) OF GLYPHOSATE APPLICATIONS TO ITS FOOD PLANT

5.1 Introduction

Glyphosate is a foliar applied broad spectrum herbicide that is translocated from the treated green growth to underground roots, rhizomes and stolons (Anon, 1999). Because the active ingredient is translocated through the plant, arthropods feeding from glyphosate-treated plants, such as phytophagous Heteroptera, may be affected by possible changes in nutritional status of the host plant.

Like many plant-feeding insects, phytophagous Heteroptera inject enzyme-rich saliva into plant tissue to break the starch down into sap (Miller, 1971). *Leptopterna dolabrata* (L.) (Heteroptera: Miridae), a grass-feeding bug commonly found in more damp field margins (Southwood & Leston, 1959), feeds exclusively on several grass species and exploits high nitrogen levels when the plant is actively growing (McNeill, 1973) by feeding from the mesophyll cells (McNeill, 1971). Since glyphosate-treated plants start to lose their vigour as the herbicide takes effect, it is possible that the high levels of nitrogen required by *L. dolabrata* will fall as the host plant senescences.

In order to identify possible indirect effects of glyphosate applications to non-target habitat on *L. dolabrata*, this experiment aimed to record any disruptions in quality of food plants treated with different rates of glyphosate, using mortality rates as an indicator of food quality.

5.2 Materials & Methods

Thirty individual plants of *Dactylis glomerata* L. were raised from native seed (Pope & Chapman, Bishops Stortford, UK) in 10cm pots of John Innes No2 compost in early February 1997. The plants were stored in a frost-free poly-tunnel (approximately 30m x 6m x 3m high) and watered as necessary. In order to ensure that the morphology, physiology and surface properties of the pot-grown plants were as close to field-grown plants as possible (Davies & Blackman, 1989) and therefore reduce possible differences in response of the plants to glyphosate (Garrod, 1989), the plants were moved to an external, enclosed area once the likelihood of severe frost had passed in mid-May.

This experiment was conducted prior to obtaining the results from the dose response chapter (Chapter 4), thus the dose rates of glyphosate were not based on dose-response of *D. glomerata*. One of three treatments was applied to each of ten plants on 29.vi.1997: a water control, 180g and 360g glyphosate ha⁻¹ (Round Up Biactive, Monsanto, High Wycombe, UK) using a precision pot sprayer (custom built for Harper Adams University College by J. Reader) fitted with Lurmark 110° flat fan nozzles (03-F110, Longstanton, Cambridge, UK), delivering a volume rate of 200 litres ha⁻¹. The plants were allowed to dry for one hour, before being placed in a well ventilated poly-tunnel until the bugs were introduced two days later, in order to ensure that the heteropteran bugs did not come into direct contact with the aquatic solution of the glyphosate compound.

Third and fourth instar *L. dolabrata* were collected using a sweep net from *D. glomerata* plants along unsprayed grassy verges close to Harper Adams University College, Shropshire (grid reference SJ 712204) on 30.vi.1997. The juveniles were stored over-night

in large plastic boxes containing fresh *D. glomerata* and sealed with muslin cloth 'lids' outdoors in a sheltered location.

Seven juvenile *L. dolabrata* were introduced to each plant on 1.vii.1997, and the plants were then enclosed with muslin cloth to prevent escape by the bugs. The plants were returned to the poly-tunnel and juveniles were classified as either dead or alive at 5, 10 and 15 days post-spray application. The plants were placed in the poly-tunnel since laboratory restrictions did not allow live insects to be used in controlled environment units. It was also considered that adverse weather conditions (wind and heavy rainfall occurred during part of this experiment) would dislodge the muslin tents and heavy rain might cause the bugs to adhere to, and become entangled in the fine weave of the cloth due to water surface tension.

5.2.1 Statistical Analysis

The percent mortality data were arc-sine transformed to assume normality and regression analysis was used to test for relationships between mortality in each of the treatments and time after spray application. Separate one-way analyses of variance, with rate of glyphosate as main effect, was used to determine whether mortality differed between treatments at 5, 10 and 15 days after spray application. Where differences were significant, contrast analysis was used as an *a priori* comparison to determine where the differences lay (Sokal & Rohlf, 1995).

5.3 Results

Mean percent mortality of *L. dolabrata* on food plants in each of the three treatments significantly increased with time after glyphosate application (Table 5.1, Figure 5.1).

Table 5.1. Relationship between percent mortality of *Leptopterna dolabrata* on food plants treated with water, 180g and 360g glyphosate ha⁻¹ and time after spray application.

Control				180g				360g			
a	b	r ²	P	a	b	r ²	P	a	b	r ²	P
8.56	22.35	0.58	0.001	19.69	22.62	0.58	0.001	24.23	21.51	0.56	0.001

Significant values at *P*<0.05 in bold.

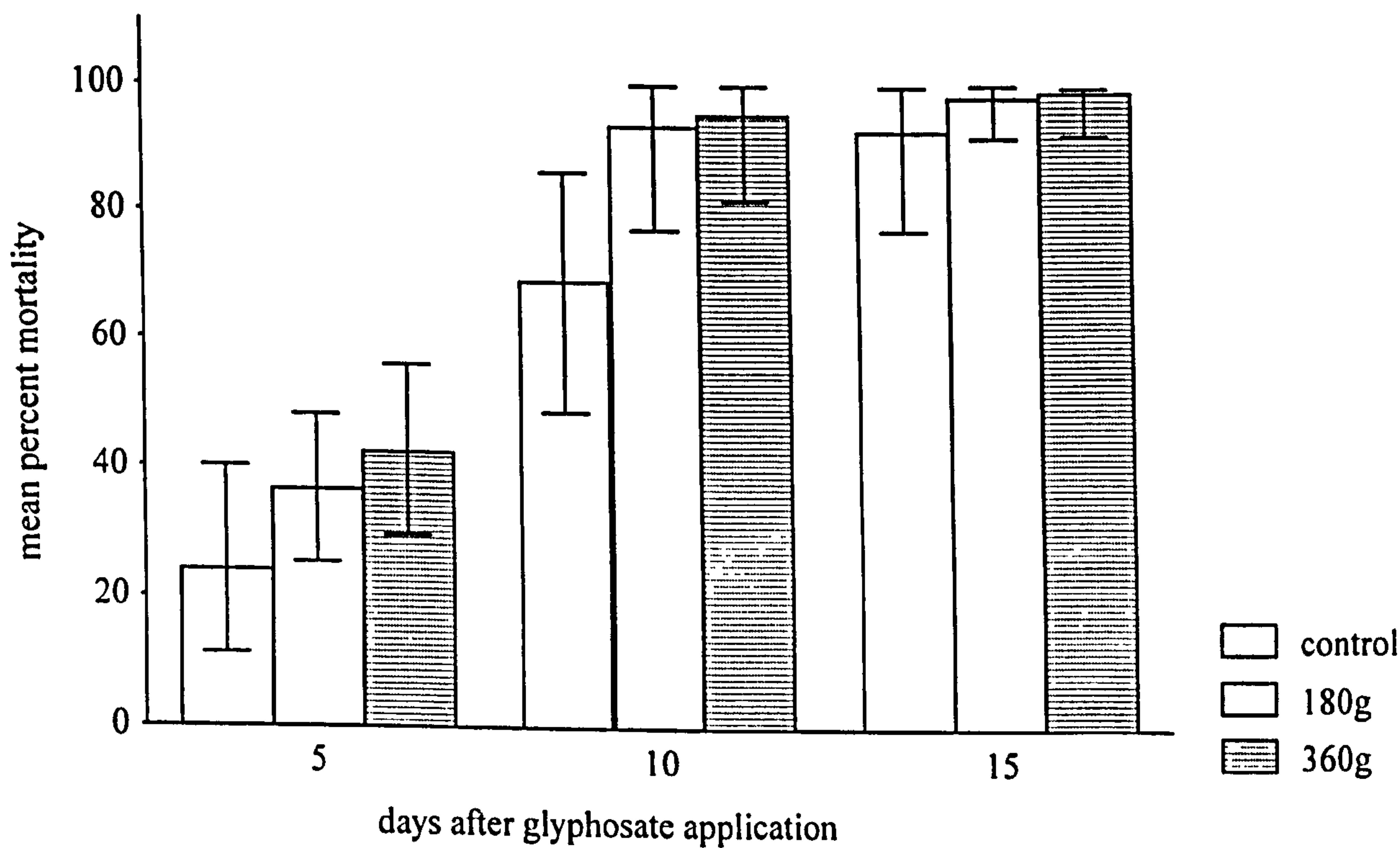


Figure 5.1. Back-transformed mean percent mortality of *Leptopterna dolabrata* and 95% confidence limits in control, 180g and 360g glyphosate ha⁻¹ treatments at 5, 10 and 15 days after spray application.

Mortality of juvenile *L. dolabrata* did not vary significantly between treatments at 5 and 15 days after glyphosate application, however, there were significant differences in mortality at 10 days post-spray (Table 5.2, Figure 5.1). Contrast analysis indicated that mortality in the glyphosate treatments was significantly higher than in the control (Table 5.3).

Table 5.2. One-way ANOVA of percent mortality of *Leptopterna dolabrata* in treatments at 5, 10 and 15 days after spray application and treatment.

Days	$F_{(2, 27)}$	P
5	2.47	0.104
10	4.22	0.025
15	1.18	0.321

Significant values at $P < 0.05$ in bold.

Table 5.3. Contrast analysis of percent mortality of *Leptopterna dolabrata* in the control, 180g and 360g glyphosate ha⁻¹ treatments at 10 days after spray application.

	$F_{(1, 27)}$	P
control v 180g	5.67	0.025
control v 360g	6.93	0.014
180g v 360g	0.06	0.804

Significant values at $P < 0.05$ in bold.

5.4 Discussion

Mortality of juvenile *L. dolabrata* was significantly greater on food plants sprayed with both rates of glyphosate at 10 days after treatment only: at 5 and 15 days after treatment there were no significant differences in mortality. However, despite the apparent differences in mortality, there was a significant increase in mortality with time within each treatment. The combination of increasing mortality with time in all treatments (including

the control) and the absence of differences in mortality between treatments at 5 and 15 days post-spray application suggests there was an underlying sensitivity of the bugs to handling and/or the experimental conditions that became more apparent than any effect of the glyphosate on the food plants towards the end of the experiment. Indeed, control mortality was unacceptably high and increased from 24.2% at 5 days post-spray application to 92.5% at 15 days. Such high rates of control mortality may have obscured any true pattern of effect of glyphosate applications to food plants on juvenile *L. dolabrata*.

McNeill (1973) also found rearing nymphs of *L. dolabrata* difficult especially since the field population of *L. dolabrata* under investigation was density-dependent upon two factors, one of which was increased temperatures. Furthermore, McNeill (1973) suggested that reductions in humidity, due to increases in temperature, cause death at moulting due to enhanced dehydration. Although temperature in the poly-tunnel was not recorded, it is likely that great fluctuations in both temperature and humidity contributed to the high control mortalities.

Apparently little published work regarding the effect of pesticide applications to food plants exists, however, Brust (1990) did not find any differences in mortality between carabids that had consumed glyphosate-treated and untreated prey, suggesting that glyphosate was non-toxic. However, this experiment, although tentative, suggests that feeding behaviour in *L. dolabrata* could be disrupted, because significantly fewer juveniles died feeding from the untreated *D. glomerata* than from the treated plants at 10 days after spray application. One possible cause for this could be a reduction in nitrogen-availability as the treated plants lost their vigour and the ability to photosynthesise as efficiently as untreated plants. Maturing juveniles require high levels of nitrogen for egg and sperm production (McNeill & Southwood, 1978) and change their feeding habit accordingly from

leaf-feeding to seed feeding to exploit the shifting nitrogen status in the anatomy of the host plant (McNeill, 1971). In this experiment, the bugs had access to the entire food plant, which included ripening seed-heads, therefore it is hypothesised that individuals possibly already weakened by low levels of humidity (due to inappropriate experimental conditions) were affected by insufficient nutrition caused by glyphosate.

Due to laboratory constraints it was not possible to use a controlled environment unit, which would have allowed regulation of temperature, humidity and lighting conditions, and may have reduced the control mortality of the heteropteran bugs. It is recommended that the experiment be repeated, once appropriate handling and experimental conditions are determined, in order to determine whether the results from this experiment are an indication of the true impact of glyphosate applications to food plants on the feeding behaviour and mortality of juvenile *L. dolabrata*.

6 TOXICITY OF GLYPHOSATE TO TWO SPECIES OF ARTHROPOD

6.1 Introduction

Toxicity testing of agricultural pesticides on non-target and beneficial arthropods is required under the European Directive 91/414/EEC for the registration of new plant protection products (Anon, 1991) and is enforced in the UK by the Plant Protection Products Regulations 1995 (Anon, 1995c).

The Society of Environmental Toxicology and Chemistry (Europe) published guidelines (Barrett *et al.*, 1994) for the laboratory, semi-field and field testing of pesticides on non-target arthropods in response to the 91/414/EEC Directive and the guidelines provide a recommended list of species for screening. Test arthropods for chemicals to be used in arable crops include the parasitoids *Aphidius rhopalosiphi* (Insecta: Hymenoptera: Aphidiidae) and *Trichogramma cacoeciae* (Insecta: Hymenoptera: Trichogrammatidae), the ground dwelling predators *Poecilus cupreus* L. (Insecta: Coleoptera: Carabidae), *Pardosa* spp (Arachnida: Araneae: Lycosidae) and *Aleochara bilineata* (Insecta: Coleoptera: Staphylinidae) and the foliage-dwelling predators *Episyrphus balteatus* (Insecta: Diptera: Syrphidae), *Chrysoperla canea* (Insecta: Neuroptera: Chrysopidae) and *Coccinella septempunctata* (Insecta: Coleoptera: Coccinellidae) (Barrett *et al.*, 1994). This list comprises selected key beneficial arthropods and clearly other beneficial, non-target, and innocuous arthropods are absent from the testing procedures.

There are two methods of testing the toxicity of pesticides on non-target arthropods in the laboratory: i) topical application and, ii) overspray. The former has the advantage of applying a measured quantity of active ingredient to each target, thus allowing identification of inherent susceptibility to the active ingredient of the pesticide. The main

disadvantage of this technique is that it does not represent a realistic field exposure.

The overspray technique replicates the field application volume and presents a worst case scenario in terms of exposure. For this reason, the method is advocated for pesticide registration procedures using the highest recommended dose (Barrett *et al.*, 1994) although the results may over-predict the likely effects of the pesticide in a field situation.

Förster *et al.*, (1997) recommend the testing of many doses of the pesticide under investigation as a method of obtaining dose-response data, however the problem of developing an adequate risk assessment model for individual species still remains. Risk assessment for individual species is almost impossible due to the existence of large numbers of species, therefore, it is more practical to do risk assessments for specific areas and the representative main communities. Risk models need to include data on size of the treated area, frequency with which the pesticide is applied, the toxicity of the product, dispersion behaviour of the arthropod and the reproductive capability of the population (Förster *et al.*, 1997).

The abundance of non-target arthropods has been shown to be significantly reduced following applications of herbicide to cropped and non-cropped areas (Chiverton & Sotherton, 1991; Feber *et al.*, 1995). Glyphosate is an increasingly widely used herbicide and, in the UK, 635 tonnes of active ingredient were applied to arable land in 1998 (D. Garthwaite, pers. comm.) compared with 276 tonnes in 1996 (Thomas *et al.*, 1997). Because grassy arable field margins are exposed to direct and misplaced applications of herbicide, including glyphosate, the effect of glyphosate on non-target arthropods in field margins is of interest. Studies on the direct toxic effects of glyphosate on arthropods, other than those recommended for registration purposes, have been limited since laboratory tests

have shown general low toxicity (Hassan *et al.*, 1988). Thus, in order to establish whether glyphosate has a toxic effect on field margin arthropod species relevant to this particular study, glyphosate was screened against two common field margin species of arthropod representative of groups forming the main subjects of the field experiments (see chapter 7).

The toxicity of glyphosate, at doses up to and including the highest recommended field dose in the UK (Anon, 1999b) was tested on adult female *Lepthyphantes tenuis* (Blackwall) (Araneae: Linyphiidae) and adults and juveniles of *Leptopterna dolabrata* (L.) (Heteroptera: Miridae). Both species occur frequently in grassy arable field margins and represent different feeding strategies. *L. tenuis* is an important arable pest predator (Sunderland *et al.*, 1986; Alderweireldt, 1994), while *L. dolabrata* is a non-target, grass-feeding (Southwood & Leston 1959) chick food item.

6.2 Materials and Methods

6.2.1 Test Compound

Technical grade glyphosate (Monsanto, Louvain-La Neuve, Belgium) was used rather than formulated glyphosate in order to isolate possible toxic effects of the glyphosate and eliminate any possible effects of formulation products. The technical grade glyphosate, present as 62% w.v. isopropylamine salt, was formulated on the day of each experiment with distilled water to make 2160g, 1440g, 1080g, 720g, 360g and 180g glyphosate ha⁻¹ treatments, (equivalent to 6L, 4L, 3L, 2L, 1L and 0.5L glyphosate ha⁻¹). Distilled water was used as a control.

6.2.2 Exposure Chambers

Plastic cups (7cm diameter x 9cm high) were used as exposure chambers with silica sand as the inert substrate. The cups were filled with 95g sand (96.32% SiO₂; Bathgate Silica Sand Ltd., Sandbach, UK), which was moistened with distilled water to 70% of its water holding capacity (250g sand : 48g water, F. Tencalla, pers. comm.). In order to contain arthropods within the chambers and to allow ventilation of pesticide vapours, muslin cloth covered each chamber and was held in place with a rubber band.

6.2.3 Arthropods

The arthropods were collected from wild populations rather than reared, since arthropod rearing is a complex exercise and only relatively few individuals were required for one instance. Insect rearing is only useful when large numbers of targets are required for long periods of time (Cannon, 1989). Furthermore, spiders used for regulatory testing are always hand-collected (F. Tencalla, pers. comm.).

L. dolabrata was collected using a sweep net from its food-plant *Dactylis glomerata* L. (Cocksfoot) along grassy verges on 27 & 28.vi.1999. *L. tenuis* was collected using a modified garden-vac suction sampler (Ryobi RSV3100E, Ryobi Outdoor Products Inc., Chandler, Arizona, USA) from grazed pasture on 12 & 13.vii.1999. Both collection areas had not been sprayed with herbicide and were close to Harper Adams University College, Shropshire (grid reference SJ 712204).

The captured arthropods were transferred immediately to the exposure chambers (one individual per chamber) in a controlled environment cabinet (Environmental Control

System CMP 3244, Winnipeg, Manitoba, Canada RSH OR9) set at 85% relative humidity, photo-period of 16 hours light : 8 hours dark and temperature 19°C:12°C until spray application the following day. Individuals of *L. dolabrata* were provided with seed heads of the food-plant *D. glomerata*, while *L. tenuis* was not provided with food as it is known that spiders can survive for weeks without food (Nakamura, 1987; Weyman *et al.*, 1994).

In this experiment, 20 adult female and 20 juvenile *L. dolabrata* and 40 female *L. tenuis* were used for each treatment, and each treatment was applied to the arthropods once. Adult female and juvenile 3rd and 4th instar *L. dolabrata* were used to detect any differences in response between the stages of maturity. Only adult female *L. tenuis* were selected since it is impossible to identify juveniles in the field. Single sexes of the spiders and Heteropteran bugs were used since there is evidence to suggest that there may be a differential response between males and females (e.g. Dinter, 1996) and females were used due their local abundance. Although testing the response of both sexes is desirable, the constraints of both time and facilities limited experimental work to just one sex.

6.2.4 Glyphosate Application Method

The direct overspray method was used for this experiment, as used in ecotoxicological testing for registration of pesticides in Europe (Barrett *et al.*, 1994), as it allows replication of the worst case scenario likely to be encountered by arthropods in a field situation.

The exposure chambers (with the muslin cloth 'lid' removed) containing moistened sand and one arthropod were placed in a precision pot sprayer (custom built for Harper Adams University College by J. Reader) and the glyphosate was applied at a field rate of 200 litre ha⁻¹, spray pressure 2.5 bar using Lurmark 110⁰ flat fan nozzles (03-F110, Longstanton,

Cambridge, UK). The control of distilled water was applied first, followed by the lowest concentration of glyphosate working up to the highest rate. The sprayer was rinsed with distilled water between applications to avoid contamination of treatments. Immediately after spray application, the muslin cloth 'lids' were replaced and the exposure chambers were returned to the controlled environment.

6.2.5 Arthropod Monitoring

The arthropods were monitored every 12 hours up to 72 hours post-spray application and were categorised as either alive or dead.

6.2.6 Statistical Analysis

Kendall's coefficient of rank correlation (tau) (Sokal & Rohlf, 1995) was used to test for association between percentage mortality of individuals and rate of glyphosate at 24, 48 and 72 hours after the spray application.

6.3 Results

6.3.1 Adult *Leptopterna dolabrata*

No dose-related mortality was seen throughout the experiment. At 24 hours, there was 0% mortality in the control, 1080g and 2160g ha⁻¹ dose rates. Maximum mortality of 15% was obtained at 720g ha⁻¹. At 48 hours after the spray application mortality had increased and was observed in each treatment, where maximum mortality of 30% occurred in the control. At 72 hours mortality had increased to 46.4% and 40% in the 180g and 2160g treatments respectively and was greater than 20% in the other treatments (Figure 6.1).

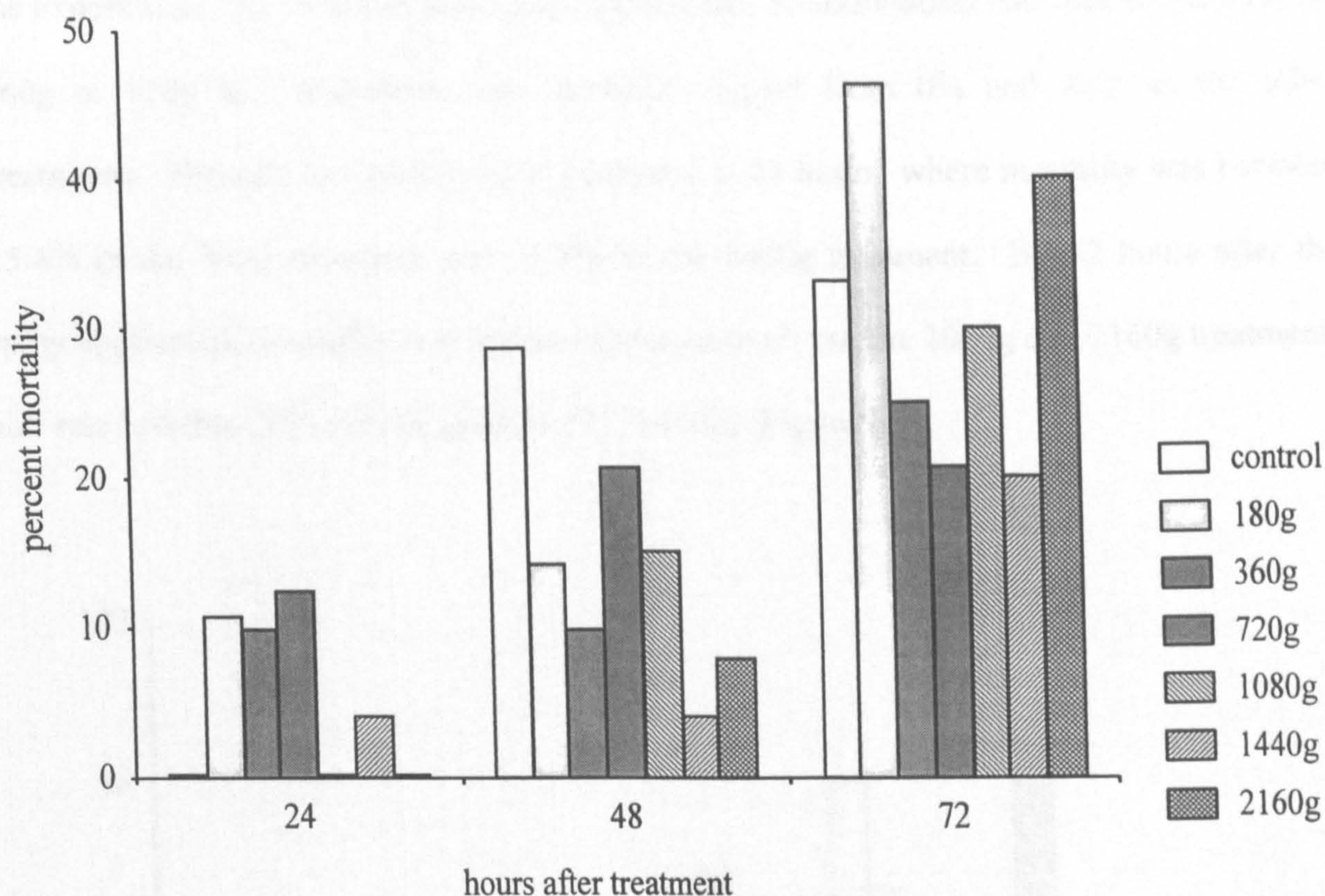


Figure 6.1 Percent mortality of adult *Leptopterna dolabrata* in control and 180g, 360g, 720g, 1080g, 1440g and 2160g glyphosate ha⁻¹ treatments at 24, 48 and 72 hours after spray application.

There was no significant association between rate of glyphosate and percentage mortality of adult *L. dolabrata* at any time after the spray application (Table 6.1).

Table 6.1. Kendall's test for association between rate of glyphosate and percent mortality in adult *Leptopterna dolabrata* at 24, 48 and 72 hours after spray application.

Hours	Tau	P
24	-0.21	0.516
48	-0.52	0.099
72	-0.24	0.453

6.3.2 *Leptopterna dolabrata* Nymphs

Mortality of *L. dolabrata* nymphs was generally greater than that of the adults throughout

the experiment. At 24 hours post spray application, no individuals had died in the control, 360g or 720g ha⁻¹ treatments, but mortality ranged from 0% and 40% in the other treatments. Nymphs had died in each treatment at 48 hours, where mortality was between 15.4% in the 360g treatment and 68.8% in the 1440g treatment. By 72 hours after the spray application, mortality had further increased in all but the 1080g and 2160g treatments and was between 20% (2160g) and 87.5% (1440g) (Figure 6.2).

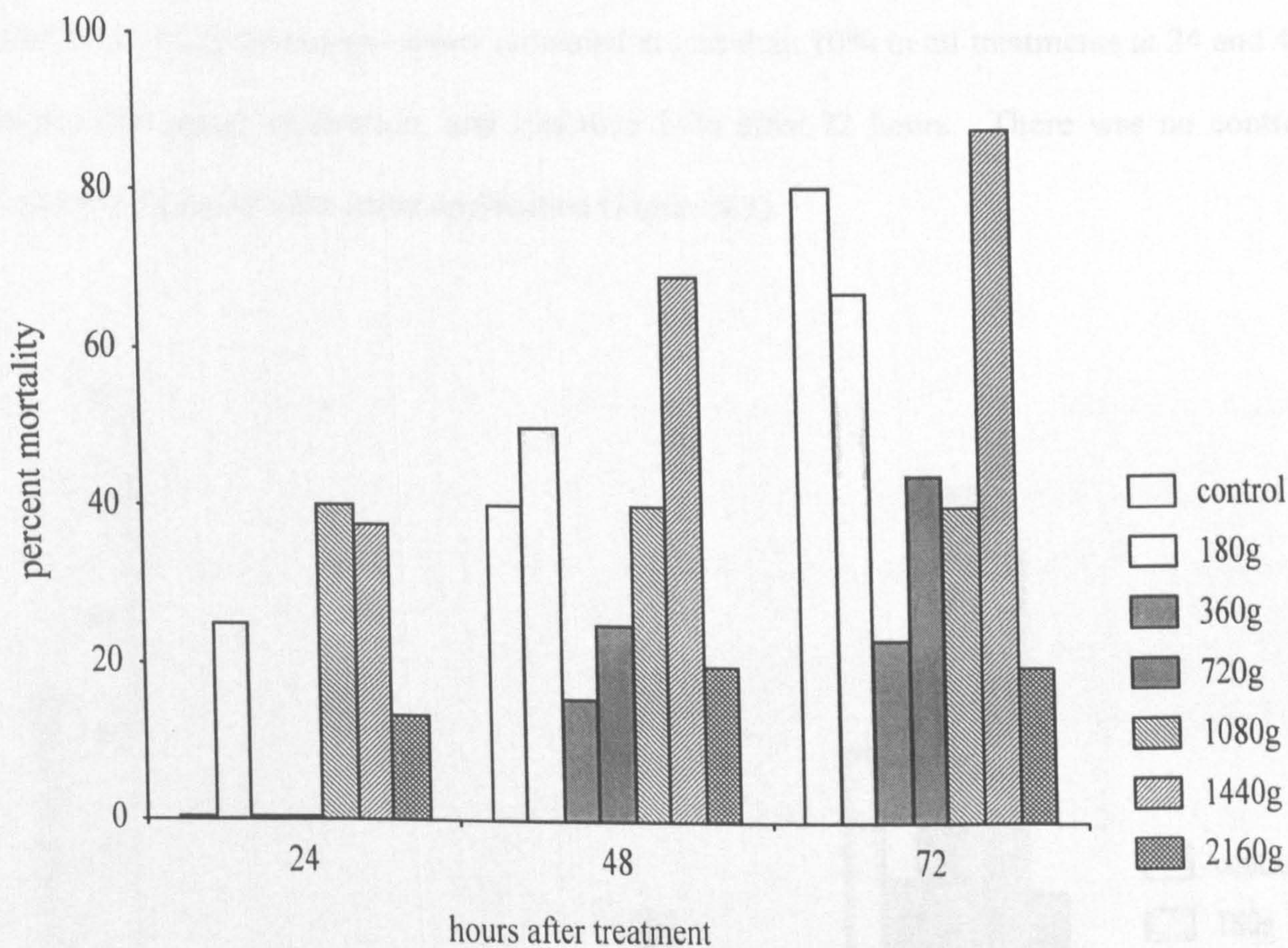


Figure 6.2 Percent mortality of *Leptopterna dolabrata* nymphs in control and 180g, 360g, 720g, 1080g, 1440g and 2160g glyphosate ha⁻¹ treatments at 24, 48 and 72 hours after spray application.

Kendall's test for association showed that there was no significant association between percent mortality of *L. dolabrata* nymphs and rate of glyphosate at any time after spray application (Table 6.2).

Table 6.2. Kendall's test for association between rate of glyphosate and percent mortality in *Leptopterna dolabrata* nymphs at 24, 48 and 72 hours after spray application.

Hours	Tau	P
24	0.31	0.333
48	0.00	1.000
72	-0.33	0.293

6.3.3 *Lepthyphantes tenuis*

Mortality of *Lepthyphantes tenuis* remained at less than 10% in all treatments at 24 and 48 hours after spray application, and less than 14% after 72 hours. There was no control mortality 72 hours after spray application (Figure 6.3).

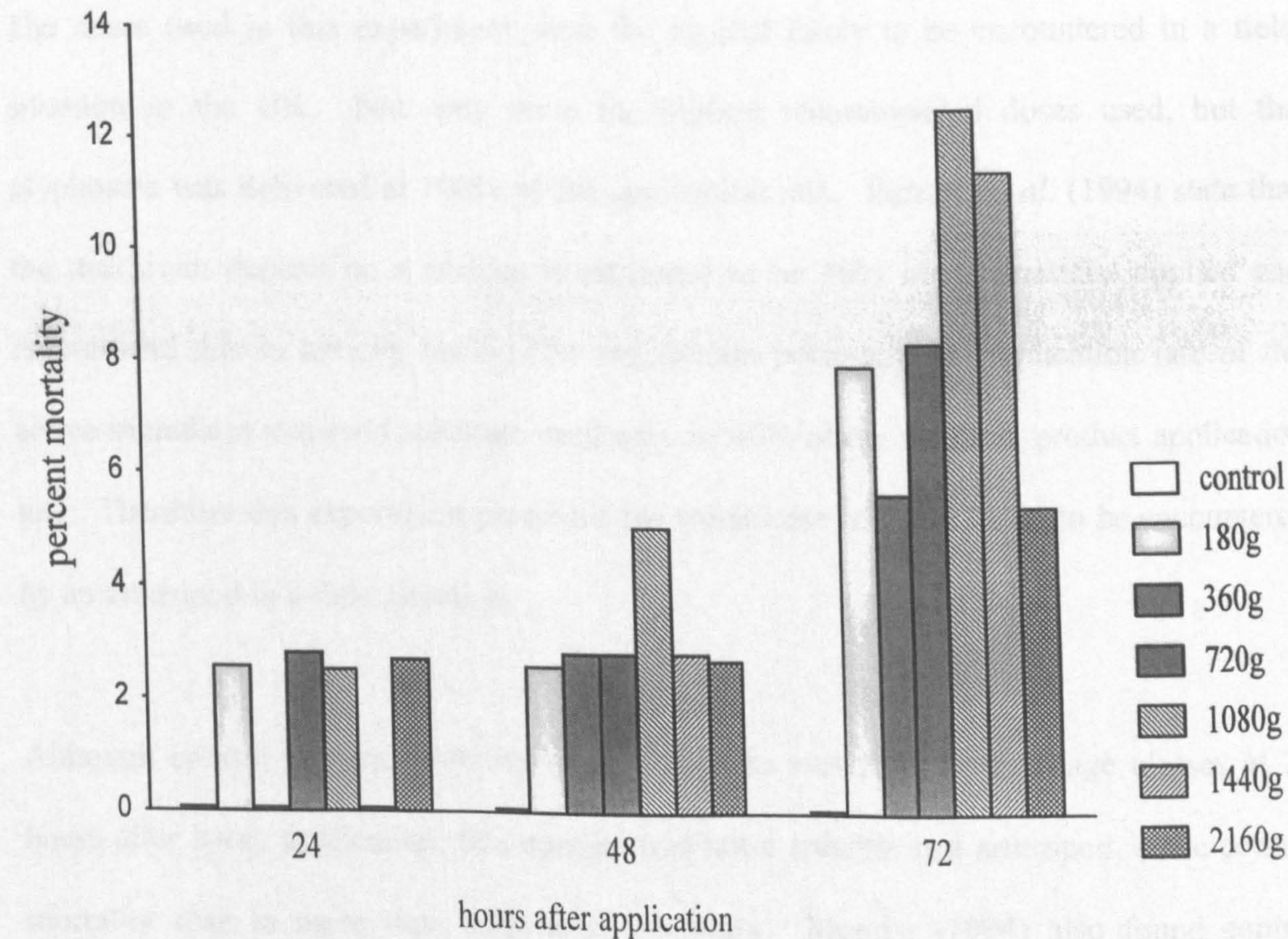


Figure 6.3 Percent mortality of *Lepthyphantes tenuis* in control and 180g, 360g, 720g, 1080g, 1440g and 2160g a.i. glyphosate ha⁻¹ treatments at 24, 48 and 72 hours after spray application.

Mortality of *L. tenuis* was not associated with glyphosate. Table 6.3 presents Kendall's coefficient for correlation between percent mortality of *L. tenuis* and rate of glyphosate treatment at 24, 48 and 72 hours after spray application.

Table 6.3. Kendall's test for association between rate of glyphosate and percent mortality in *Lepthyphantes tenuis* at 24, 48 and 72 hours after spray application.

Hours	Tau	P
24	0.21	0.516
48	0.41	0.194
72	0.33	0.293

6.4 Discussion

The doses used in this experiment were the highest likely to be encountered in a field situation in the UK. Not only were the highest recommended doses used, but the glyphosate was delivered at 100% of the application rate. Barrett *et. al.* (1994) state that the maximum deposit on a surface is estimated to be 40% of the material applied and recommend that in toxicity testing (for registration purposes) the application rate of the active ingredient to a sand substrate need only be 40% of the maximal product application rate. Therefore this experiment presented the worse case scenario likely to be encountered by an arthropod in a field situation.

Although control percent mortality of *L. dolabrata* was zero for both age classes at 24 hours after spray application, this species was not a suitable test arthropod, since control mortality rose to more than 30% after 24 hours. Moreby (1994) also found control mortality at 24 hours post spray application to be low (<25%) for a species of Miridae (Heteroptera: *Calocoris norvegicus*).

There were no significant associations between increasing rate of glyphosate and percent mortality of either adult or juvenile *L. dolabrata* at any time after spray application. Indeed, high control mortalities of both adult and juvenile *L. dolabrata* suggest that factors other than glyphosate contributed to mortality. These factors probably relate to the sensitivity of *L. dolabrata* to handling and inappropriate confinement conditions, including inadequate food supply.

Lepthyphantes tenuis was more tolerant to the test conditions, since control mortality for the spiders was zero. Despite control mortality of *L. tenuis* remaining at zero throughout the experiment, there was no significant association between increasing rate of glyphosate and percent mortality of female *L. tenuis* where mortality remained at less than 14%. Furthermore, mortality of *L. tenuis* was well below 30% which is the level at which testing for regulatory purposes classifies an active ingredient as harmful (Anon, 1997). Thus, glyphosate appeared to be harmless to female *L. tenuis*.

The results for female *L. tenuis* indicate that glyphosate, even when applied at the highest recommended field rate, is non-toxic and therefore the spiders would be unaffected by glyphosate applications in a field situation. These data support previous glyphosate toxicity data, where it has been shown to be harmless to *Calocoris norvegicus* (Heteroptera: Miridae) (Moreby, 1994), *Anthocoris nemoralis* (Heteroptera: Anthocoridae) and *Chiracanthium mildei* (Araneae: Clubionidae) (Hassan *et al.*, 1988).

In order to conclusively establish whether glyphosate is toxic to *L. dolabrata* and *L. tenuis*, however, this experiment needs refining to produce more robust data for statistical analysis and, to reduce control mortality of *L. dolabrata*. By maintaining the number of individuals tested and increasing the replication of the experiment from just one to at least five, more

appropriate analysis techniques such as probit analysis may be applied. Handling and maintenance conditions for *L. dolabrata* need to be explored and refined so that control mortality may be reduced. This would involve providing living food-plant material, altering temperature, humidity and light conditions and any other factors that may become apparent.

7 EFFECTS OF HERBICIDE APPLICATIONS TO A GRASSY ARABLE FIELD MARGIN ON NON-TARGET ARTHROPODS

7.1 Introduction

Grassy arable field margins are important habitats for arthropods in agro-ecosystems, since they can provide a network of permanent and overwintering habitat and refuges from intensive farming practices (e.g., Thomas *et al.*, 1990; Dennis & Fry, 1992; Petit & Burel; 1998). The non-target arthropod fauna in field margins comprises not only beneficial predators (e.g., Araneae, some Carabidae; Staphylinidae) (Coombes & Sotherton, 1986; Smith *et al.*, 1993), but also important prey items for other arthropods and vertebrates (e.g., Heteroptera, Homoptera, Lepidoptera and Symphyta larvae) (Chiverton, 1999).

Due to their proximity to areas of high agrochemical input, arable field margins are exposed to direct and indirect applications of herbicide. Exposure to herbicides has been shown to reduce weed cover and diversity in the field margins (de Snoo, 1994; de Snoo & van der Poll, 1999). The effects of intentional and misplaced herbicide applications to field margins on non-target arthropods has been little studied (e.g. Smith *et al.*, 1993; Feber *et al.*, 1995), however, data from herbicide treated and untreated cereal headlands suggest that populations of arthropods may be affected (Raatikainen & Huhta, 1968; Hassall *et al.*, 1992; Moreby & Southway, 1999). Glyphosate has little or no insecticidal properties (Chapter 6; Moreby, 1994), therefore effects on arthropods are thought to be indirect.

This experiment investigates the effects of different rates of glyphosate applications (simulating drift and direct application rates) to grassy arable field margins on the abundance and community structure of Araneae, Carabidae and Heteroptera. These groups of arthropod represent predatory and phytophagous feeding strategies, therefore, the effects on the three groups associated with changes in vegetation may be complex.

7.2 Methods

7.2.1 Sites

Two well established 2m wide grassy arable field margins were selected on the Allerton Research and Educational Trust Estate in Leicestershire (Grid Reference SK 789015), for the experiments carried out in 1997 and 1998. In 1997, the field margin was east-south-east facing and dominated by Couch-grass (*Elymus repens* (L.)) and False Oat-grass (*Arrhenatherum elatius* (L.)). In 1998, the field margin was orientated between the west-north-west and south-south-west and dominated by False Oat-grass (*A. elatius* (L.)) and Yorkshire Fog (*Holcus lanatus* (L.)). Both field margins lay on slightly stoney clay soils from the Hanslope Series (Hodge *et al.*, 1984) adjacent to a dense uncut Hawthorn (*Crataegus monogyna* Jacq.) and Blackthorn (*Prunus spinosa* L.) hedge and the fields were sown to winter barley (cultivar: Fighter in 1997; cultivar: Regina in 1998).

7.2.2 Treatments

Each of the two lengths of field margin was divided into 32 contiguous plots measuring 12m long x 2m wide and eight replicates of four treatments were assigned in a randomised block design. In 1997 the treatments were 90g, 180g & 360g glyphosate ha⁻¹ (Roundup Biactive, Monsanto, High Wycombe, Berkshire) and an unsprayed control (also referred to in the text as 0g - 360g glyphosate). In 1998, the treatments were 360g, 720g & 1080g glyphosate ha⁻¹ (Roundup Biactive, Monsanto, High Wycombe, Berkshire) and an unsprayed control (also referred to in the text as 0g - 1440g glyphosate). Glyphosate was applied to the plots at a volume rate of 200 litres ha⁻¹ and a pressure of 2.5 bar using an Oxford Precision Sprayer fitted with flat fan nozzles on 30.v.1997 and 4.vi.1998 during dry, calm conditions (Table 7.1).

Table 7.1. Conditions at spraying.

Year	Temperature	Relative Humidity	Wind
1997	24° C	55%	<1 m s ⁻¹
1998	17.5° C	84.5%	<2 m s ⁻¹

7.2.3 Arthropod Sampling

Epigeal arthropods were sampled using a modified garden-vac (g-vac) (Ryobi RSV3100E, Ryobi Outdoor Products Inc., Chandler, Arizona, USA). The arthropod samples from each experimental plot comprised 10 sub-samples of 30 second sucks at 1m intervals along each experimental plot. Sampling was done on the central 10m of each plot to avoid edge effects from neighbouring treatments and the total sampling area per plot approximated to 0.13m². Each sample of arthropods was emptied from the g-vac into a plastic bag, which was sealed and immediately placed into a cool box containing frozen freezer packs to reduce arthropod activity and hence, predation.

All Araneae, Carabidae and Heteroptera were extracted with an aspirator into 70% alcohol and adult Araneae, Carabidae and Heteroptera were identified to species level. Heteroptera and Carabidae were identified by the author using Southwood & Leston (1959) and Lindroth (1996) respectively and Araneae were identified by James Bell (Department of Biology, Roehampton Institute) using Roberts (1985; 1987).

7.2.4 Vegetation Sampling

Percentage ground cover of dead vegetation in the experimental plots was recorded using permanent 0.25m² quadrats and average vegetation height at five positions (domino-5)

within the quadrats was recorded to the nearest centimetre. The quadrats were positioned at 3m - 3.5m, 6m - 6.5m and 9m - 9.5m.

7.2.5 Sampling Programme

Arthropod and vegetation sampling was done two weeks subsequent to spray application and monthly thereafter to monitor any changes over the season. Thus, sampling ran from June - October inclusive. In 1998, the unsprayed control and 360g glyphosate ha⁻¹ treatments from the 1997 experiment were re-sampled in May and September to identify any long term effects of glyphosate application. Arthropod and vegetation sampling and identification were done as detailed above.

7.2.6 Statistical Analysis

All arthropod data were $\log x + 1$ transformed to assume normality, whereas all raw vegetation data were normally distributed. Univariate repeated measures ANOVA (von Ende, 1993), with date as the within-subject factor and treatment as the main effect, was used to analyse differences in arthropod abundance throughout the season since samples were not wholly independent of each other. Mauchly's W was used to test for sphericity of the data since this is an assumption of the repeated measures ANOVA (von Ende, 1993). Where there was a significant departure from sphericity, a multivariate approach to repeated measures was used, since this method does not require the spherical pattern of data (von Ende, 1993). In the event that data were spherical, but there was an interaction between date and treatment, indicating that the effect of treatment varied with date, a univariate one-way ANOVA was computed with treatment as main effect for each sample-date in order to eliminate the effect of time (von Ende, 1993).

Where there were significant differences in arthropod abundance between treatments, contrast analysis was used as an *a priori* comparison to identify where significant differences lay, as this hypothesis was implicit within the experimental design (Sokal & Rohlf, 1995).

Linear regression analysis was used to explore relationships between rate of glyphosate and i) vegetation height and, ii) amount of dead vegetation cover and also between arthropod abundance m^{-2} and the i) vegetation height and, ii) amount of dead vegetation cover. Furthermore, where there was a significant date x treatment interaction within the repeated measures ANOVA, regression analyses of variables were computed from individual sample dates.

Adult Araneae, Carabidae and Heteroptera species communities in the treatments were ordinated using detrended correspondence analysis (DCA). Ordination arranges treatments along the axes on the basis of species composition data (ter Braak, 1995). Ordination in two dimensions (two axes) produces a diagram where the treatments are represented by points in two-dimensional space, where the points that are closest together correspond to sites are similar in species composition and points that are more distant correspond to sites that are dissimilar in species composition (ter Braak, 1995). DCA was used because it avoids the arch-effect (the second axis is an arched function of the first), which is common in other ordination programs (e.g. correspondence analysis, factor analysis) without assuming that the data be multivariate normal (e.g. principal components analysis) (Gauch, 1994). An alternative method of ordination, canonical correspondence analysis (CCA) which utilises measured environmental gradients was not used because it assumes species have a unimodal distribution along environmental gradients (ter Braak, 1995), and may also be subject to the arch-effect (Palmer, 1993). Furthermore, since just two vegetation

variables were measured, this may have provided a 'noisy' incomplete environmental data set, thus it was more appropriate to ordinate the arthropod communities in relation to each other.

The DCA program, DECORANA (Hill, 1994) was used to analyse the species abundance data, where all species were included in the analysis, with no down weighting (Hill, 1994). In order to interpret the importance of the vegetation variables and rate of glyphosate on the arthropod communities, Spearman's rank correlation was used to test for association between the axis scores and these factors. Where significant correlations are found, this allows one to interpret the axis scores as a scale of environmental measurement, i.e., vegetation height, cover by dead vegetation or rate of glyphosate as either an increasing or decreasing scale, according to the nature of the association (negative or positive). This method avoids the problems of the CCA program, CANOCO, where interpretation of the axes to represent gradients is not possible (M. Palmer, pers. comm., Department of Botany, Oklahoma State University, USA).

Spearman's rank correlation was also used to test for association between rate of glyphosate and vegetation height in the plots sampled 12 and 16 months after the glyphosate application. Correlation analysis was used since the effects of only two rates of glyphosate on arthropod abundance m^{-2} were investigated.

Computations of all statistics, with the exception of the DCA, were done using Statistica (StatSoft, 1999).

7.3 Results

7.3.1 Vegetation

Species composition in the experimental field margins was dominated by grasses, especially *Elymus repens* and *Arrhenatherum elatius*, with the occasional occurrence of forbs, including *Stachys sylvatica* and *Cirsium arvense*. There were no changes in species composition over the sampling season, but there were changes in vegetation height and amount of dead vegetation in the plots. Refer to Appendix 1 for a list of plant species recorded from the experimental field margin plots in 1997 and 1998 combined.

Analysis of vegetation height and percentage dead vegetation cover data in the experimental grassy margins showed a significant interaction between sample date and glyphosate treatment in both sample years (i.e., 1997 & 1998). Consequently, the effect of rate of glyphosate on these variables was analysed separately for each month in each year.

Table 7.2. Repeated measures ANOVA results for the height of vegetation (Height) and dead vegetation cover (Dead) in field margins with sampling date and treatment as main effects in 0g - 360g (1997) and in 0g - 1440g (1998) glyphosate ha⁻¹ treatments.

	0g - 360g				0g - 1440g			
	Height		Dead		Height		Dead	
	<i>F</i> _(12, 112)	<i>P</i>	<i>F</i> _(12, 112)	<i>P</i>	<i>F</i> _(12, 112)	<i>P</i>	<i>F</i> _(12, 112)	<i>P</i>
date x treatment	3.48	0.001	7.65	0.001	13.77	0.001	3.87	0.001

Significant values at *P* < 0.05 in bold.

Figures 7.1 and 7.2 illustrate mean vegetation height from June to October in plots treated with 0g - 360g (1997) and 0g - 1440g (1998) glyphosate ha⁻¹ respectively. Mean percentage dead vegetation cover in plots treated with 0g - 360g (1997) and 0g - 1440g (1998) glyphosate ha⁻¹ over the same period is illustrated in Figures 7.3 and 7.4 respectively.

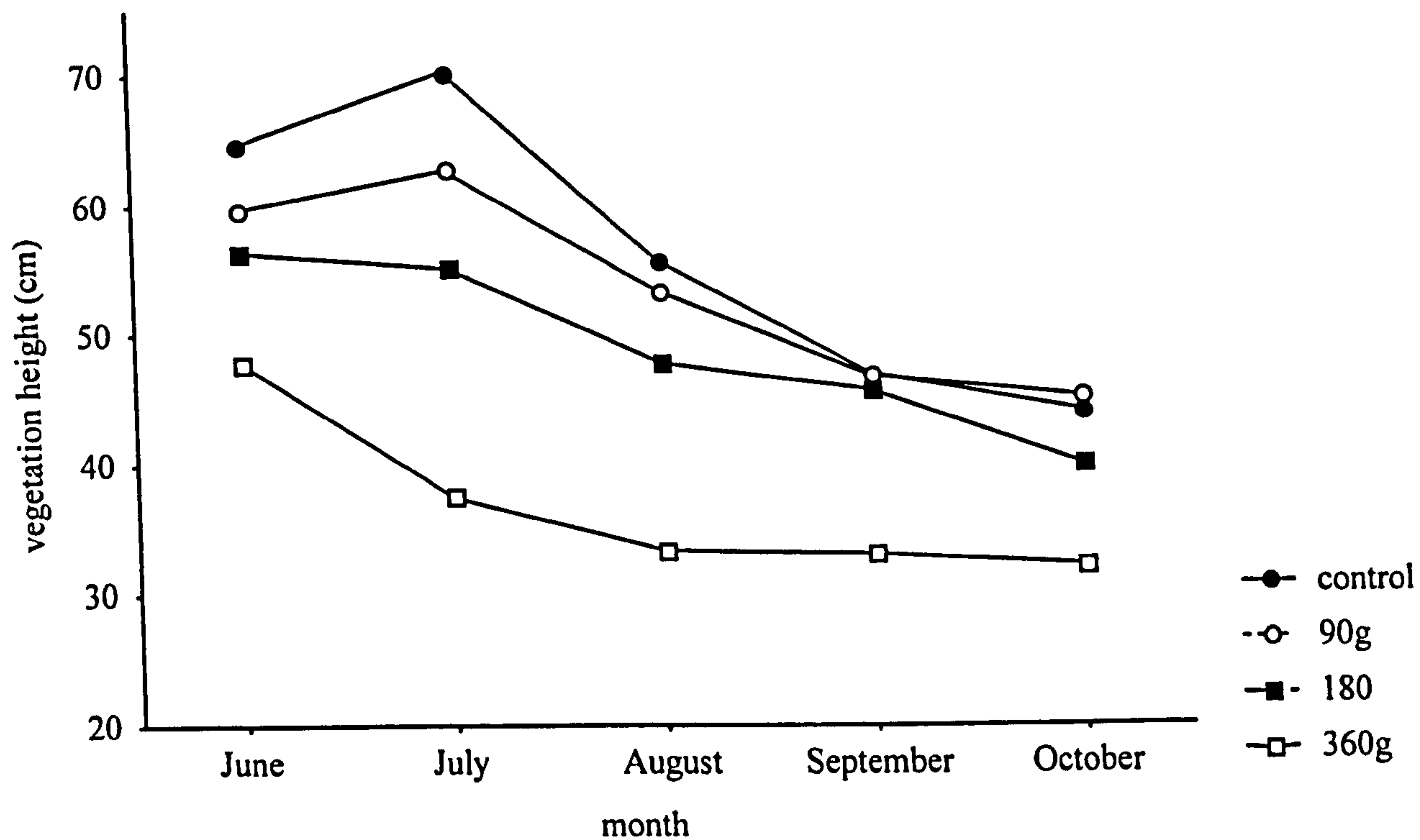


Figure 7.1. Mean vegetation height in unsprayed control (0g), 90g, 180g and 360g glyphosate ha⁻¹ treatments in each sample month (1997).

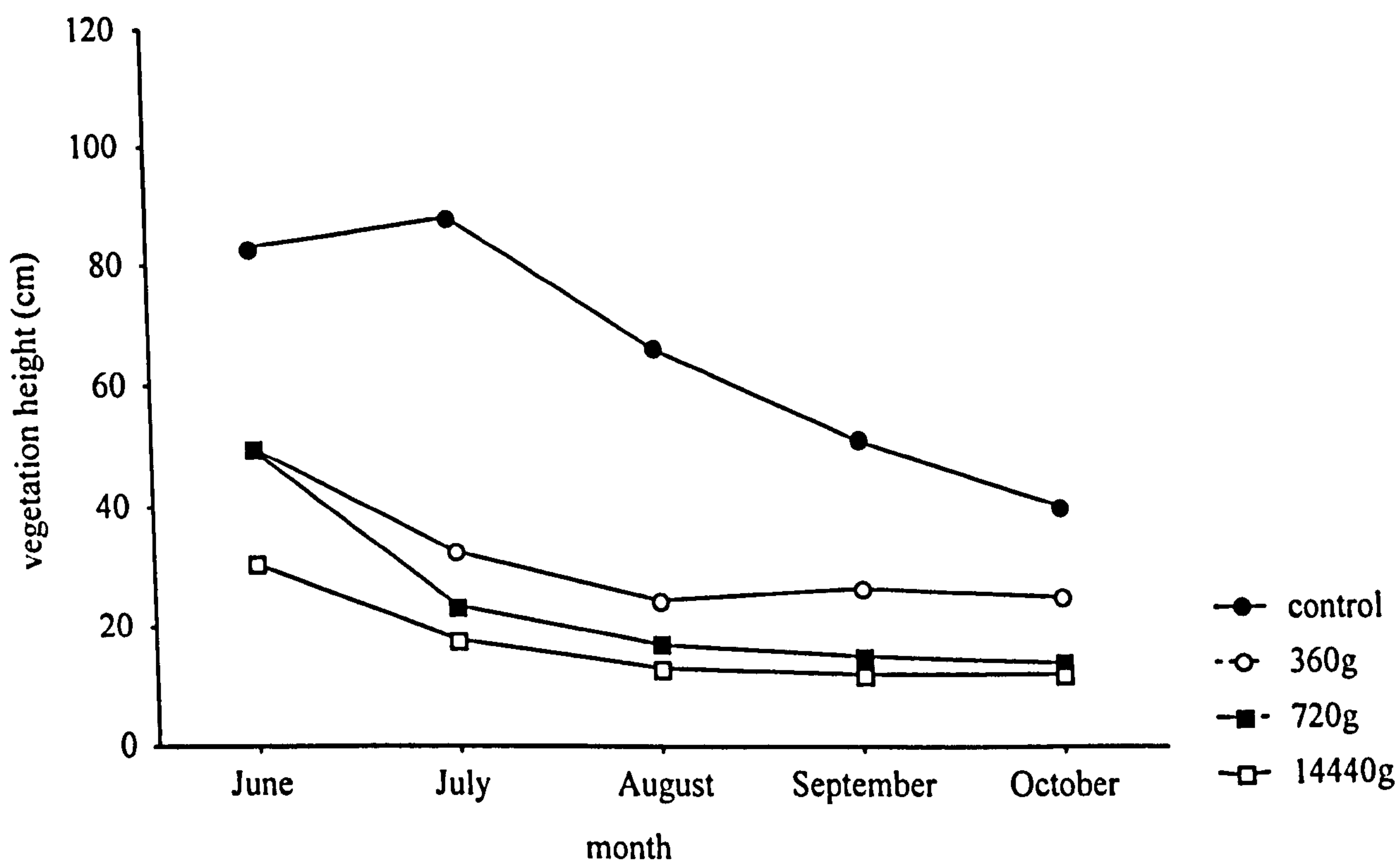


Figure 7.2. Mean vegetation height in unsprayed control (0g), 360g, 720g and 1440g glyphosate ha⁻¹ treatments in each sample month (1998).

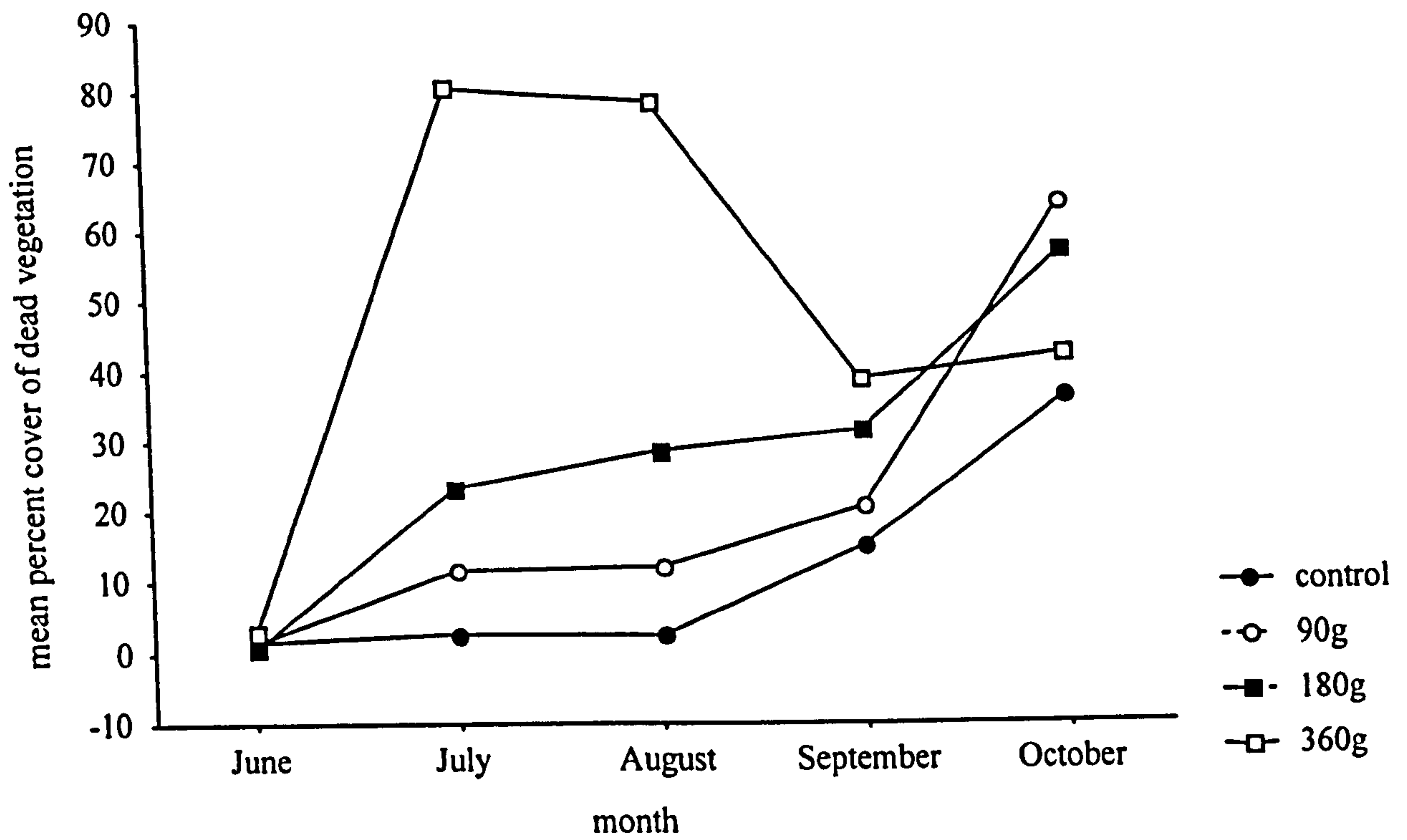


Figure 7.3. Mean percentage dead vegetation cover in unsprayed control (0g), 90g, 180g and 360g glyphosate ha⁻¹ treatments in each sample month (1997).

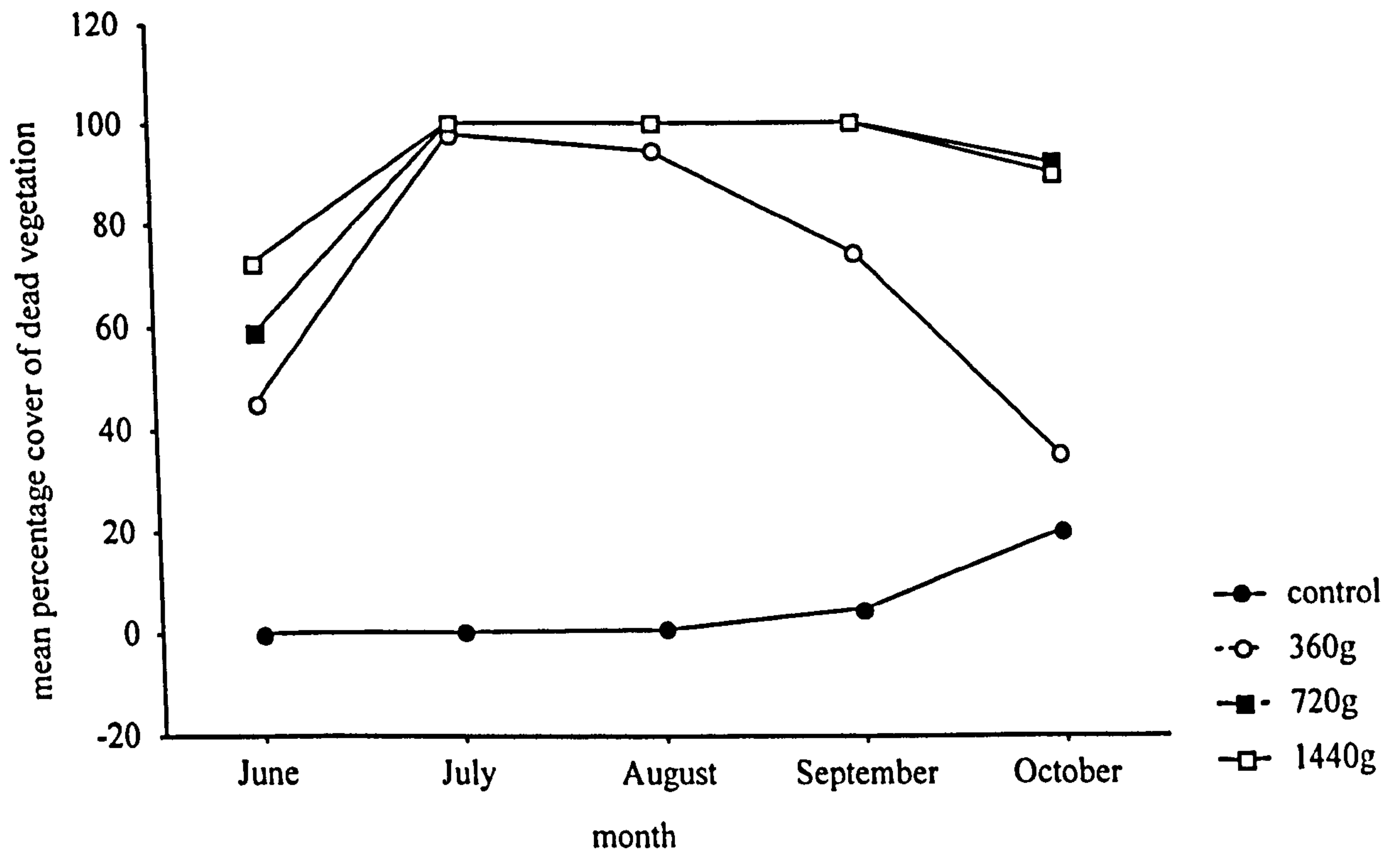


Figure 7.4. Mean percentage dead vegetation cover in unsprayed control (0g), 360g, 720g and 1440g glyphosate ha⁻¹ treatments in each sample month (1998).

There was a significant negative linear relationship between rate of glyphosate and the height of vegetation from July to October in both years of the experiment (Table 7.3). Rate of glyphosate was also positively related to the amount of dead vegetation cover in July and August at rates of up to 360g ha⁻¹ used in 1997. However, this relationship existed in every month when rates of up to 1440g glyphosate ha⁻¹ used in 1998 (Table 7.3).

Table 7.3. Regression analysis of effect of rate of glyphosate on vegetation height (Height) and amount of dead vegetation (Dead) in experimental plots in each month.

Month	Regression parameters	0g – 360g (1997)		0g - 1440g (1998)	
		Height	Dead	Height	Dead
June	a	64.28	1.10	72.30	19.81
	b	-0.05	0.01	-0.03	0.04
	r ²	0.27	0.05	0.53	0.26
	<i>P</i>	0.002	0.237	0.001	0.003
July	a	70.63	-5.17	57.55	39.14
	b	-0.09	0.22	-0.03	0.06
	r ²	0.53	0.72	0.43	0.48
	<i>P</i>	0.001	0.001	0.001	0.001
August	a	57.56	-3.71	42.99	48.83
	b	-0.06	0.22	-0.03	0.05
	r ²	0.52	0.61	0.49	0.41
	<i>P</i>	0.001	0.001	0.001	0.001
September	a	49.34	24.51	47.06	29.67
	b	-0.04	0.04	-0.03	0.06
	r ²	0.32	0.03	0.57	0.58
	<i>P</i>	0.001	0.343	0.001	0.001
October	a	46.02	53.37	40.60	14.40
	b	-0.04	0.01	-0.02	0.06
	r ²	0.28	0.01	0.56	0.60
	<i>P</i>	0.002	0.716	0.001	0.001

Significant values at *P*<0.05 in bold.

7.3.2 Arthropods

The unit of abundance of arthropods referred to in this section (7.3) is the number of individuals per m². The abundances of Araneae, Carabidae and Heteroptera in the control and 360g glyphosate ha⁻¹ treatments in 1997 and 1998 were compared to determine whether it would be possible to analyse all treatments over the two years as a continuum of rates of glyphosate. There were significantly more individuals sampled in 1997 than in 1998 (Table 7.4), therefore analyses were undertaken for each year separately, i.e., 0g - 360g (1997) and 0g - 1440g (1998) glyphosate ha⁻¹.

Table 7.4. One-way ANOVA results for differences in abundance m⁻² of Araneae, Carabidae and Heteroptera in the unsprayed control and 360g glyphosate ha⁻¹ treatments in 1997 and 1998.

	1997		1998		<i>F</i> _(1, 158)	<i>P</i>
	mean	SE ±	mean	SE ±		
Araneae	31.15	2.95	23.54	2.07	4.47	0.036
Carabidae	12.08	1.31	7.93	0.71	7.73	0.006
Heteroptera	26.66	2.81	16.26	1.80	9.71	0.002

Significant values at *P*<0.05 in bold.

7.3.2.1 Araneae - Total Abundance

The abundance of Araneae increased significantly from June to October in 1997 (0g - 360g glyphosate ha⁻¹) (*F*_(4, 112) = 18.95, *P*<0.001) and in 1998 (0g - 1440g glyphosate ha⁻¹) (*F*_(4, 112) = 11.54, *P*<0.001). However, since there was a significant date x treatment interaction in both 1997 (0g - 360g glyphosate ha⁻¹ treatments) and 1998 (0g - 1440g glyphosate ha⁻¹ treatments) (*F*_(1, 112) = 2.99, *P*<0.001; *F*_(12, 112) = 1.89, *P*<0.05 in 1997 and 1998 respectively). The effect of glyphosate treatment in individual months was analysed separately. Abundance of Araneae in the 0g - 360g glyphosate ha⁻¹ treatments significantly

differed only within the September sample (Table 7.5), where there were significantly fewer spiders in the 360g glyphosate ha⁻¹ treatment than in the unsprayed control, 90g and 180g glyphosate ha⁻¹ treatments (Table 7.6). Figure 7.5 shows the back-transformed mean abundance of spiders m⁻² with 95% confidence limits in the 0g - 360g glyphosate ha⁻¹ treatments from each sample date.

Table 7.5. One-way ANOVA results for abundance of Araneae in the unsprayed control (0g), 90g, 180g and 360g glyphosate ha⁻¹ treatments in June, July, August, September and October 1997.

	<i>F</i> (3, 28)	<i>P</i>
June	1.22	0.320
July	2.35	0.093
August	0.24	0.868
September	4.01	0.017
October	2.10	0.122
Significant values at <i>P</i> <0.05 in bold.		

Table 7.6. Contrast analysis results for differences in Araneae abundance between unsprayed control (0g), 90g, 180g and 360g glyphosate ha⁻¹ treatments in September 1997.

	<i>F</i> (1, 28)	<i>P</i>
0g v 90g	0.41	0.525
0g v 180g	0.29	0.597
0g v 360g	10.03	0.004
90g v 180g	0.01	0.915
90g v 360g	6.37	0.018
180g v 360g	6.93	0.014
Significant values at <i>P</i> <0.05 in bold.		

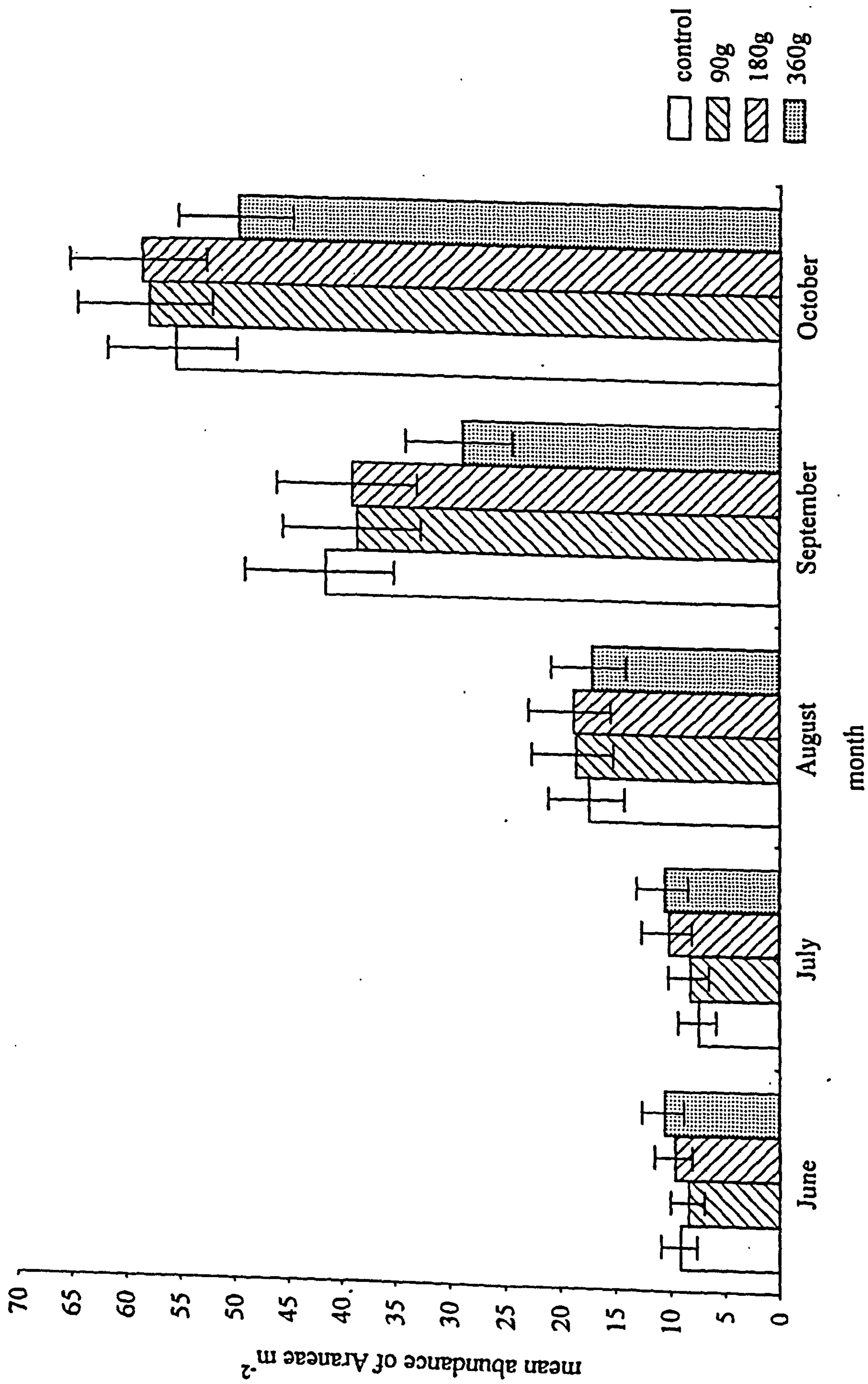


Figure 7.5. Back-transformed mean abundance of Araneae (m^{-2}) and 95% confidence limits in the unsprayed control, 90g, 180g and 360g glyphosate ha^{-1} treatments in each sample month.

In 1998, Araneae abundance in the 0g - 1440g glyphosate ha⁻¹ treatments significantly differed in the samples from July to October inclusive (Table 7.7). There were significantly fewer Araneae in the 360g glyphosate ha⁻¹ treatment than in the unsprayed control in July and October and abundance was significantly lower in the 720g glyphosate ha⁻¹ treatment than in the unsprayed control in September and October. The 1440g glyphosate ha⁻¹ treatment supported significantly fewer Araneae than the unsprayed control in July, August, September and October. The abundance of Araneae was significantly greater in the 360g glyphosate ha⁻¹ treatment than in the 1440g glyphosate ha⁻¹ treatment in August (Table 7.8). Figure 7.6 shows the back-transformed mean spider abundance m⁻² and 95% confidence limits in the 0g - 1440g glyphosate ha⁻¹ treatments from each sample date.

Table 7.7. One-way ANOVA of abundance of spiders in the unsprayed control (0g), 360g, 720g and 1440g glyphosate ha⁻¹ treatments in June, July, August, September and October 1998.

	<i>F</i> (3, 28)	<i>P</i>
June	0.01	0.100
July	4.82	0.008
August	4.34	0.013
September	3.28	0.036
October	5.77	0.003

Significant values at *P*<0.05 in bold.

Table 7.8. Contrast analysis results for differences in Araneae abundance between the unsprayed control (0g), 360g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

	July		August		September		October	
	<i>F</i> (1, 28)	<i>P</i>	<i>F</i> (1, 28)	<i>P</i>	<i>F</i> (1, 28)	<i>P</i>	<i>F</i> (1, 28)	<i>P</i>
0g v 360g	5.36	0.028	0.01	0.980	2.67	0.113	5.76	0.023
0g v 720g	3.58	0.069	3.31	0.080	6.65	0.015	13.45	0.001
0g v 1440g	14.18	0.001	9.11	0.005	7.97	0.009	12.49	0.001
360g v 720g	0.02	0.675	3.21	0.084	0.89	0.353	1.61	0.215
360g v 1440g	2.10	0.158	8.96	0.006	1.41	0.244	1.29	0.266
720g v 1440g	3.51	0.072	1.44	0.240	0.06	0.801	0.02	0.895

Significant values at *P*<0.05 in bold.

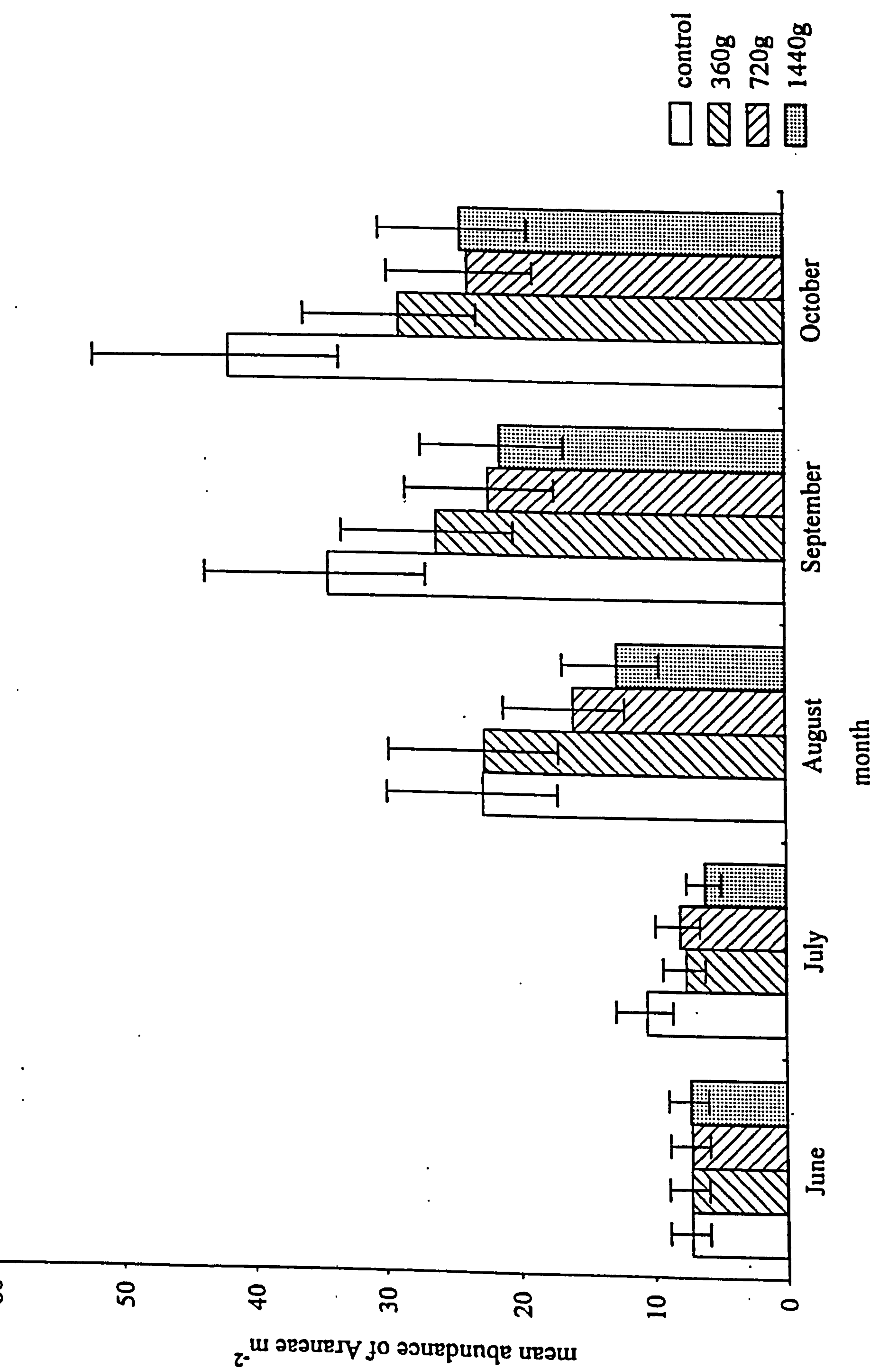


Figure 7.6. Back-transformed mean abundance of Araneae (m⁻²) and 95% confidence limits in the unsprayed control, 360g, 720g and 1440g glyphosate ha⁻¹ treatments in each sample month.

The abundance of Araneae was not related to vegetation height when glyphosate was applied at rates of less than 360g ha⁻¹. When the rates were increased to 360g, 720g and 1440g glyphosate ha⁻¹ in 1998, there was a significant positive relationship between vegetation height and abundance of Araneae in July, September and October, where vegetation height accounted for up to 53% of the variation in Araneae abundance (Table 7.9).

The amount of dead vegetation did not account for any significant variation in abundance of Araneae when rates of glyphosate of less than 360g ha⁻¹ were applied. However, when rates of glyphosate were increased to 360g, 720g and 1440g ha⁻¹ there was a significant negative relationship between the amount of dead vegetation and abundance of Araneae in July, September and October, where amount of dead vegetation accounted for up to 25% of the variation in abundance of Araneae (Table 7.9).

Table 7.20. Regression analysis of the effect of vegetation height (Height) and amount of dead vegetation (Dead) on the abundance of phytophagous Heteroptera m⁻² in 0g - 360g glyphosate ha⁻¹ (1997) and 0g - 1440g glyphosate ha⁻¹ (1998) treatments.

June			July			August			September			October		
	a	b	r ²	P	a	b	r ²	P	a	b	r ²	a	b	P
Height														
1997	0.24	0.01	0.01	0.758	-0.14	0.01	0.25	0.003	0.05	-0.01	0.05	0.82	-0.01	0.06
1998	-0.05	0.01	0.15	0.027	-0.11	0.01	0.49	0.001	-0.07	0.01	0.48	0.01	0.01	0.20
Dead														
1997	0.29	0.01	0.01	0.662	0.63	-0.01	0.35	0.001	0.26	0.01	0.01	0.58	-0.01	0.01
1998	0.09	-0.01	0.13	0.043	0.50	-0.01	0.63	0.001	0.27	-0.01	0.59	0.23	-0.01	0.32

Significant values at $P < 0.05$ in bold.

7.3.2.2 Araneae - Species Abundance

There were 82 species of Araneae from 14 families recorded over the two year sampling period (Appendix 2), where two species were present in sufficiently high densities for further analysis. *Gonatium rubens* (August - October in 1997) and *Lepthyphantes tenuis* (September & October in 1997 and June - October in 1998) (Linyphiidae) are common species of grasslands and agricultural habitats in Britain.

Abundance of *G. rubens* from August to October varied significantly between the 0g - 360g glyphosate ha⁻¹ treatments ($F_{(3, 28)} = 4.48, P < 0.05$), but there were no significant date ($F_{(2, 56)} = 1.41, P > 0.05$) or date x treatment ($F_{(6, 56)} = 0.92, P > 0.05$) effects. Abundance of *G. rubens* was significantly lower in the 180g and 360g glyphosate ha⁻¹ treatments than in the unsprayed control plots and also significantly reduced in the 360g glyphosate ha⁻¹ treatment compared with the 90g glyphosate ha⁻¹ treatment (Table 7.10). Figure 7.7 illustrates the back-transformed mean abundance of *G. rubens* m⁻² and 95% confidence limits in the 0g - 360g glyphosate ha⁻¹ treatments.

Table 7.10. Contrast analysis results for differences in abundance of *Gonatium rubens* between unsprayed control (0g), 90g, 180g and 360g glyphosate ha⁻¹ treatments in 1997.

	$F_{(1, 28)}$	P
0g v 90g	0.42	0.839
0g v 180g	4.25	0.049
0g v 360g	9.66	0.004
90g v 180g	3.44	0.074
90g v 360g	8.42	0.007
180g v 360g	1.10	0.304

Significant values at $P < 0.05$ in bold.

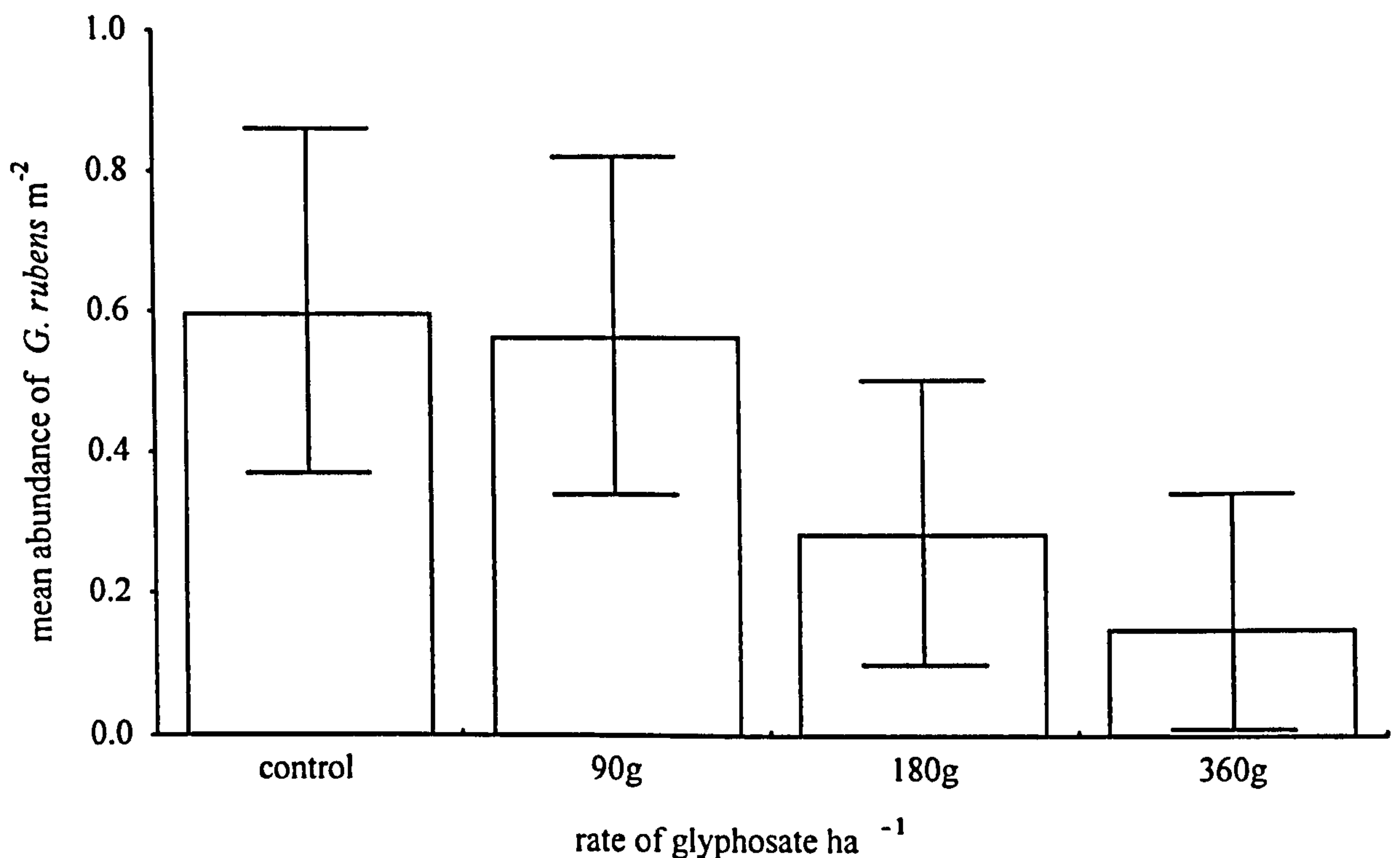


Figure 7.7. Back-transformed mean abundance of *Gonatium rubens* m⁻² and 95% confidence limits in the unsprayed control, 90g, 180g and 360g glyphosate ha⁻¹ treatments in 1997.

There was a significant positive relationship between vegetation height and abundance of *G. rubens* from August to October where it accounted for 14% of the variation ($F_{(1, 94)} = 15.71$, $P < 0.001$). Amount of dead vegetation did not affect the abundance of *G. rubens* from August to October ($F_{(1, 94)} = 0.01$, $P > 0.05$).

Abundance of *L. tenuis* varied significantly between the 0g - 360g glyphosate ha⁻¹ treatments (1997) from September to October ($F_{(3, 28)} = 7.82$, $P < 0.001$). Abundance was also significantly greater in October than in September ($F_{(1, 28)} = 399.01$, $P < 0.001$), but there was no significant date x treatment interaction ($F_{(3, 28)} = 0.43$, $P > 0.05$). Abundance of *L. tenuis* was significantly lower in the 360g glyphosate ha⁻¹ treatment than in the unsprayed control, 90g and 180g glyphosate ha⁻¹ treatments (Table 7.11). Figure 7.8

illustrates the back-transformed mean abundance of *L. tenuis* m⁻² and 95% confidence limits in the 0g - 360g glyphosate ha⁻¹ treatments.

Table 7.11. Contrast analysis results for differences in *Lepthyphantes tenuis* abundance between unsprayed control (0g), 90g, 180g and 360g glyphosate ha⁻¹ treatments in 1997.

	<i>F</i> (11, 28)	<i>P</i>
0g v 90g	0.21	0.648
0g v 180g	2.50	0.125
0g v 360g	19.42	0.001
90g v 180g	1.26	0.272
90g v 360g	15.57	0.001
180g v 360g	7.98	0.009

Significant values at *P*<0.05 in bold.

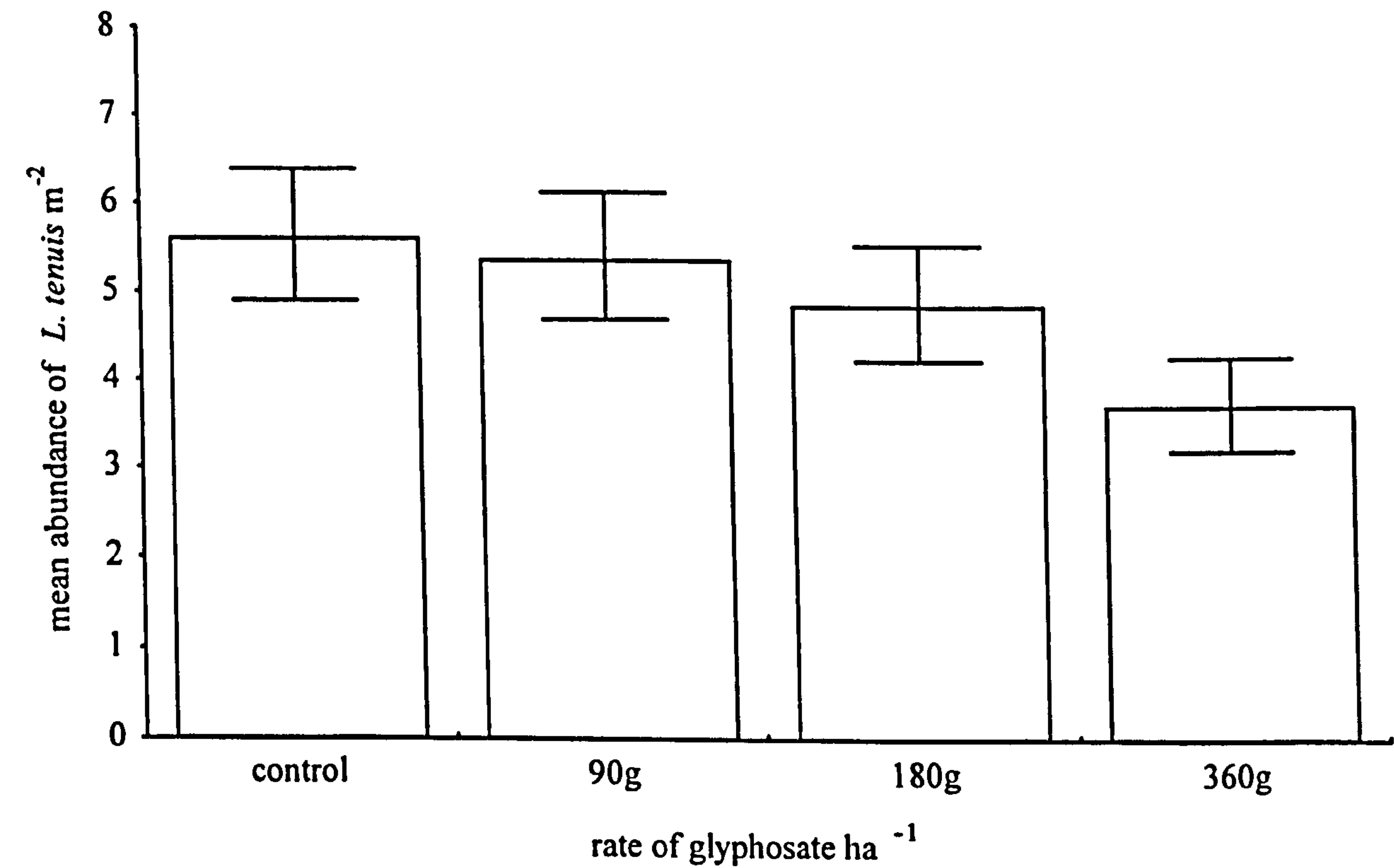


Figure 7.8. Back-transformed mean abundance of *Lepthyphantes tenuis* m⁻² and 95% confidence limits in the unsprayed control, 90g, 180g and 360g glyphosate ha⁻¹ treatments in 1997.

In 1998, although there was a significant date effect ($F_{(4, 112)} = 61.20, P<0.001$) where abundance of *L. tenuis* increased from June to October, there was no significant date x treatment interaction ($F_{(12, 112)} = 1.20, P>0.05$). However, due to a significant departure from sphericity (Mauchly's $W = 0.41, P<0.001$) within the repeated measures ANOVA of the abundance of *L. tenuis* between the 0g - 1440g glyphosate ha⁻¹ treatments, a multivariate approach to repeated measures ANOVA was adopted. Abundance of *L. tenuis* significantly varied between treatments ($R_{(15, 66)} = 4.84, P<0.001$) where abundance was significantly lower in the 360g, 720g and 1440g glyphosate ha⁻¹ treatments than in the unsprayed control. Abundance was also significantly lower in the 720g and 1440g glyphosate ha⁻¹ treatments than in the 360g glyphosate ha⁻¹ treatments (Table 7.12). Figure 7.9 illustrates the back-transformed mean abundance of *L. tenuis* m⁻² and 95% confidence limits in the 0g - 1440g glyphosate ha⁻¹ treatments.

Table 7.12. Contrast analysis results for differences in *Lepthyphantes tenuis* abundance between unsprayed control (0g), 360g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

	$R_{(15, 24)}$	P
0g v 360g	4.75	0.004
0g v 720g	13.12	0.001
0g v 1440g	15.30	0.001
360g v 720g	4.79	0.004
360g v 1440g	4.69	0.004
720g v 1440g	0.77	0.582

Significant values at $P<0.05$ in bold.

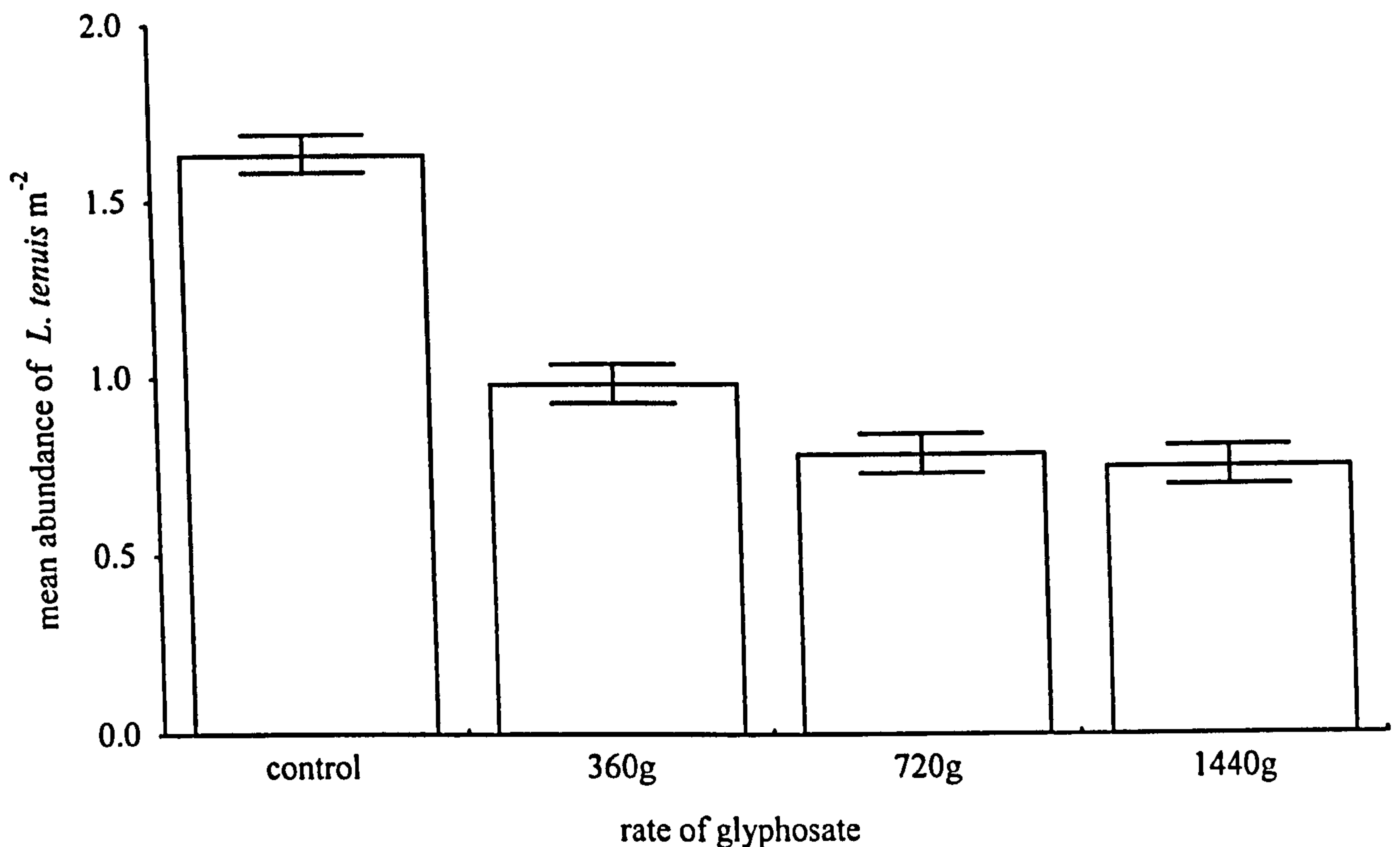


Figure 7.9. Back-transformed mean abundance of *Lepthyphantes tenuis* m⁻² and 95% confidence limits in the unsprayed control, 360g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

There was no significant relationship between vegetation height ($F_{(1, 62)} = 0.11, P > 0.05$) and abundance of *L. tenuis* when plots were treated with less than 360g glyphosate ha⁻¹ (1997) from September to October. However, there was a significant negative relationship between amount of dead vegetation and abundance ($F_{(1, 62)} = 5.86, P < 0.05, r^2 = 0.09$).

There was also no significant relationship between vegetation height and abundance of *L. tenuis* from June to October when plots were treated with up to 1440g glyphosate ha⁻¹ (1998) ($F_{(1, 158)} = 0.01, P > 0.05$). As in 1997, there was a significant negative relationship between amount of dead vegetation and abundance of *L. tenuis* from June to October 1998, accounting for 9% of the variation ($F_{(1, 158)} = 14.74, P < 0.001$).

7.3.2.3 Carabidae - Total Abundance

Abundance of Carabidae varied significantly through the 1997 (0g - 360g glyphosate ha⁻¹) sampling season ($F_{(4, 112)} = 42.55, P<0.001$), where abundance peaked in July, but there was no significant date x treatment interaction ($F_{(12, 112)} = 0.86, P>0.05$). However, due to a significant departure from sphericity (Mauchly's $W = 0.509, P<0.05$) within the repeated measures ANOVA of Carabidae abundance in the 0g - 360g glyphosate ha⁻¹ treatments, a multivariate approach to repeated measures was used. There was no significant difference in abundance of Carabidae between the 0g - 360g glyphosate ha⁻¹ treatments ($R_{(15, 66)} = 0.90, P>0.05$).

Abundance of carabid beetles significantly varied through the 1998 (0g - 1440g glyphosate ha⁻¹) sampling season ($F_{(4, 112)} = 12.14, P<0.001$), but there was no significant date x treatment interaction ($F_{(12, 112)} = 1.24, P>0.05$). Abundance of Carabidae from June to October significantly varied between the 0g - 1440g glyphosate ha⁻¹ treatments ($F_{(4, 112)} = 12.14, P<0.001$), where there were significantly greater densities of Carabidae in the unsprayed control plots than in glyphosate-treated plots (Table 7.13).

Table 7.13. Contrast analysis results for differences in Carabidae abundance between unsprayed control (0g), 306g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

	$F_{(1, 28)}$	P
0g v 360g	6.49	0.017
0g v 720g	9.77	0.004
0g v 1440g	18.11	0.001
360g v 720g	0.33	0.568
360g v 1440g	2.92	0.099
720g v 1440g	1.28	0.268

Significant values at $P<0.05$ in bold.

Figures 7.10 and 7.11 illustrate the back-transformed mean abundance of carabids and 95% confidence limits in the 0g - 360g and 0g - 1440g glyphosate ha⁻¹ treatments respectively.

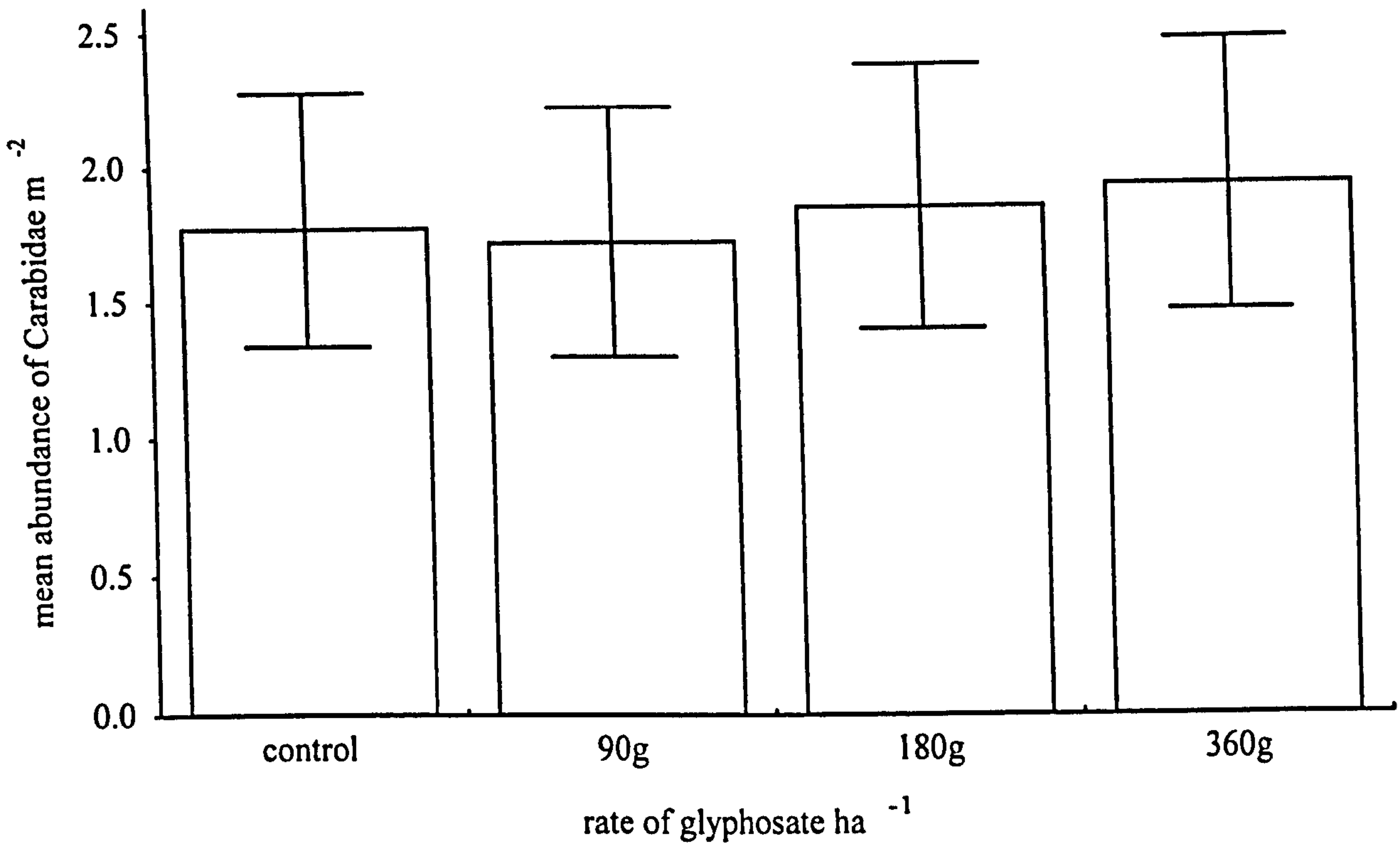


Figure 7.10. Back-transformed mean abundance of Carabidae m⁻² and 95% confidence limits in the unsprayed control, 90g, 180g and 360g glyphosate ha⁻¹ treatments in 1997.

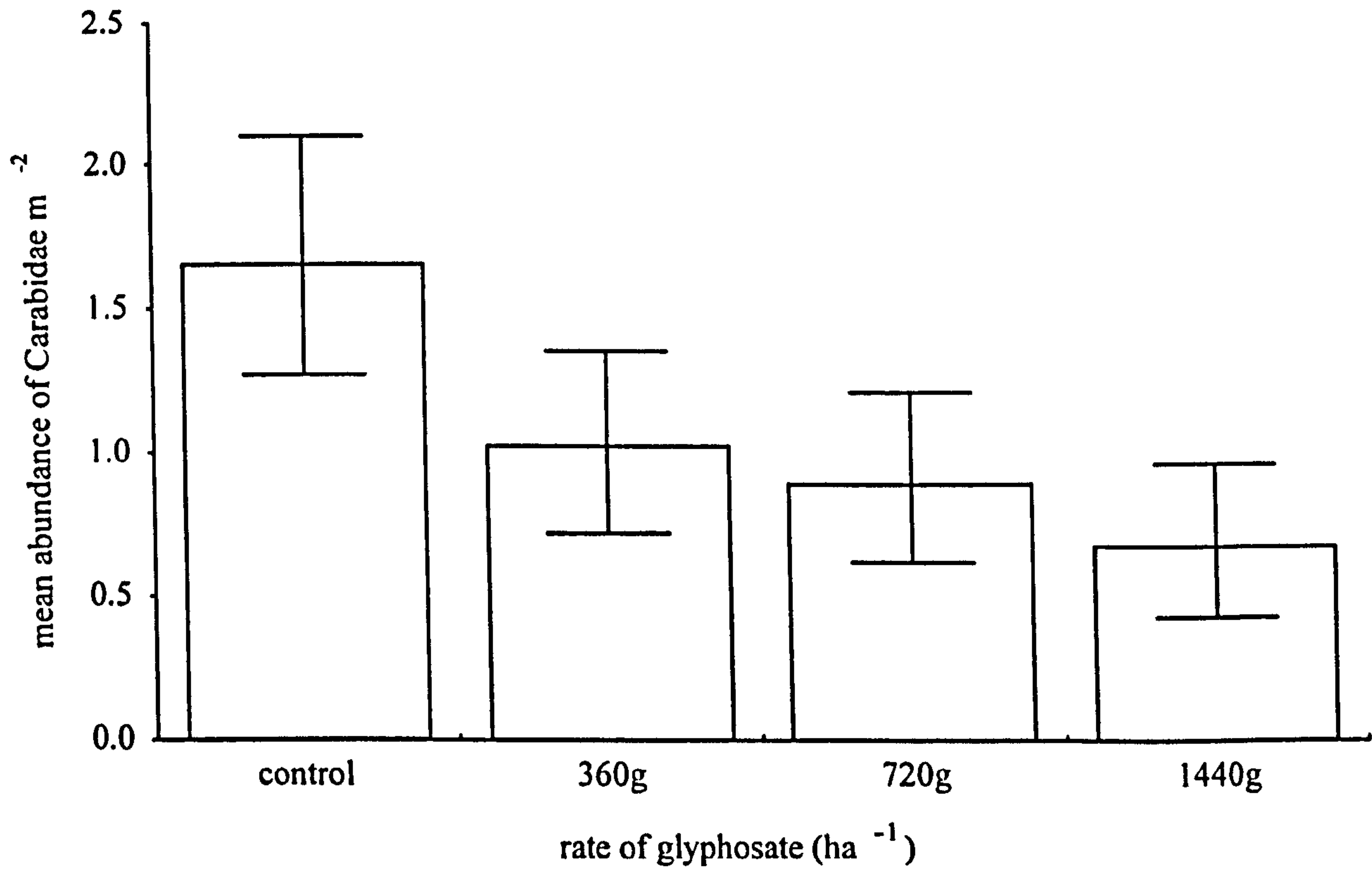


Figure 7.11. Back-transformed mean abundance of Carabidae m⁻² and 95% confidence limits in the unsprayed control, 360g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

There was no significant relationship between vegetation height and the abundance of Carabidae from June to October when rates of glyphosate of less than 360g glyphosate ha⁻¹ were applied to the field margin plots ($F_{(1, 158)} = 0.71, P > 0.05$). However, when the rates were increased to 360g, 720g and 1440g glyphosate ha⁻¹ the abundance of Carabidae from June to October was significantly positively related to the height of vegetation ($F_{(1, 158)} = 3.98, P < 0.05, r^2 = 0.02$).

The amount of dead vegetation did not affect the abundance of Carabidae from June to October when glyphosate at rates of less than 360g ha⁻¹ were applied to the field margin plots ($F_{(1, 158)} = 2.75, P > 0.05$). However, when rates were increased to 360g, 720g and 1440g, there was a significant negative relationship between amount of dead vegetation and abundance of Carabidae from June to October ($F_{(1, 158)} = 9.11, P < 0.01, r^2 = 0.05$).

7.3.2.4 Carabidae - Species Abundance

There were 24 species of Carabidae recorded from the experimental field margin plots over the two year sampling period (Appendix 3), where two species occurred at sufficiently high densities for further analysis. *Demetrias atricapillus* in October 1997 and *Trechus quadristriatus* in July 1997 (0g - 360g glyphosate ha⁻¹ treatments) and *D. atricapillus* in October 1998 (0g - 1440g glyphosate ha⁻¹ treatments). Abundance of neither *D. atricapillus* nor *T. quadristriatus* in 1997 significantly varied between treatments ($F_{(3, 28)} = 0.99, P > 0.05, F_{(3, 28)} = 0.21, P > 0.05$ for *D. atricapillus* and *T. quadristriatus* respectively) and abundance of *D. atricapillus* in 1998 did not significantly vary between the treatments ($F_{(3, 28)} = 1.55, P > 0.05$).

There were no significant relationships between vegetation height and abundance of either *D. atricapillus* or *T. quadristriatus*, or between amount of dead vegetation and abundance of either *D. atricapillus* or *T. quadristriatus* in field margins treated with up to 360g glyphosate ha⁻¹. However, when the rates were increased to 360g, 720g and 1440g glyphosate ha⁻¹, there was a significant positive relationship between vegetation height and abundance of *D. atricapillus* and a significant negative relationship between amount of dead vegetation and abundance of *D. atricapillus* (Table 7.14).

Table 7.14. Regression analysis of effect of vegetation height (Height) and amount of dead vegetation (Dead) on the abundance m⁻² of *Demetrias atricapillus* and *Trechus quadristriatus* in 0g - 360g glyphosate ha⁻¹ (97) treatments and *Demetrias atricapillus* in 0g - 1440g glyphosate ha⁻¹ (98) treatments.

	Height				Dead			
	a	b	r ²	P	a	b	r ²	P
<i>D. atricapillus</i> 97	0.83	0.01	0.01	0.878	0.92	-0.01	0.01	0.920
<i>D. atricapillus</i> 98	0.26	0.01	0.14	0.036	0.70	-0.01	0.13	0.046
<i>T. quadristriatus</i> 97	1.69	-0.01	0.01	0.920	1.66	0.01	0.01	0.962

Significant values at *P*<0.05 in bold.

7.3.2.5 Heteroptera - Total Abundance

The abundance of Heteroptera varied significantly in the 1997 sampling season (0g - 360g glyphosate ha⁻¹) (*F*_(4, 112) = 172.78, *P*<0.001) where abundance decreased from June to October, however, abundance did not differ significantly between the treatments (*F*_(3, 28) = 0.81, *P*>0.05). There was also no significant date x treatment interaction (*F*_(12, 112) = 0.64, *P*>0.05).

In 1998, abundance of Heteroptera varied significantly through the 1998 sampling season (0g - 1440g glyphosate ha⁻¹) (*F*_(4, 112) = 225.29, *P*<0.001), where abundance again

decreased from June to October. There was no significant date x treatment interaction ($F_{(12, 112)} = 0.96, P>0.05$), however, due to a significant departure from sphericity (Mauchly's $W = 0.509, P<0.05$) within the repeated measures ANOVA of abundance of Heteroptera in the 0g - 1440g glyphosate ha⁻¹ treatments, a multivariate approach to repeated measures was used. The abundance of Heteroptera varied significantly between treatments ($R_{(15, 66)} = 2.50, P<0.01$) where abundance was significantly greater in the unsprayed control than in the 360g, 720g and 1440g glyphosate ha⁻¹ treatments (Table 7.15).

Table 7.15. Contrast analysis results for differences in Heteroptera abundance between unsprayed control (0g), 306g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

	$R_{(5, 24)}$	P
0 v 360g	2.78	0.040
0 v 720g	4.25	0.007
0 v 1440g	8.13	0.001
360g v 720g	0.326	0.892
360g v 1440g	2.47	0.062
720g v 1440g	1.30	0.299

Significant values at $P<0.05$ in bold.

Figures 7.12 and 7.13 illustrate back-transformed mean abundances of Heteroptera m⁻² and 95% confidence limits in the 0g, - 360g glyphosate treatments and in the 0g - 1440g glyphosate ha⁻¹ treatments.

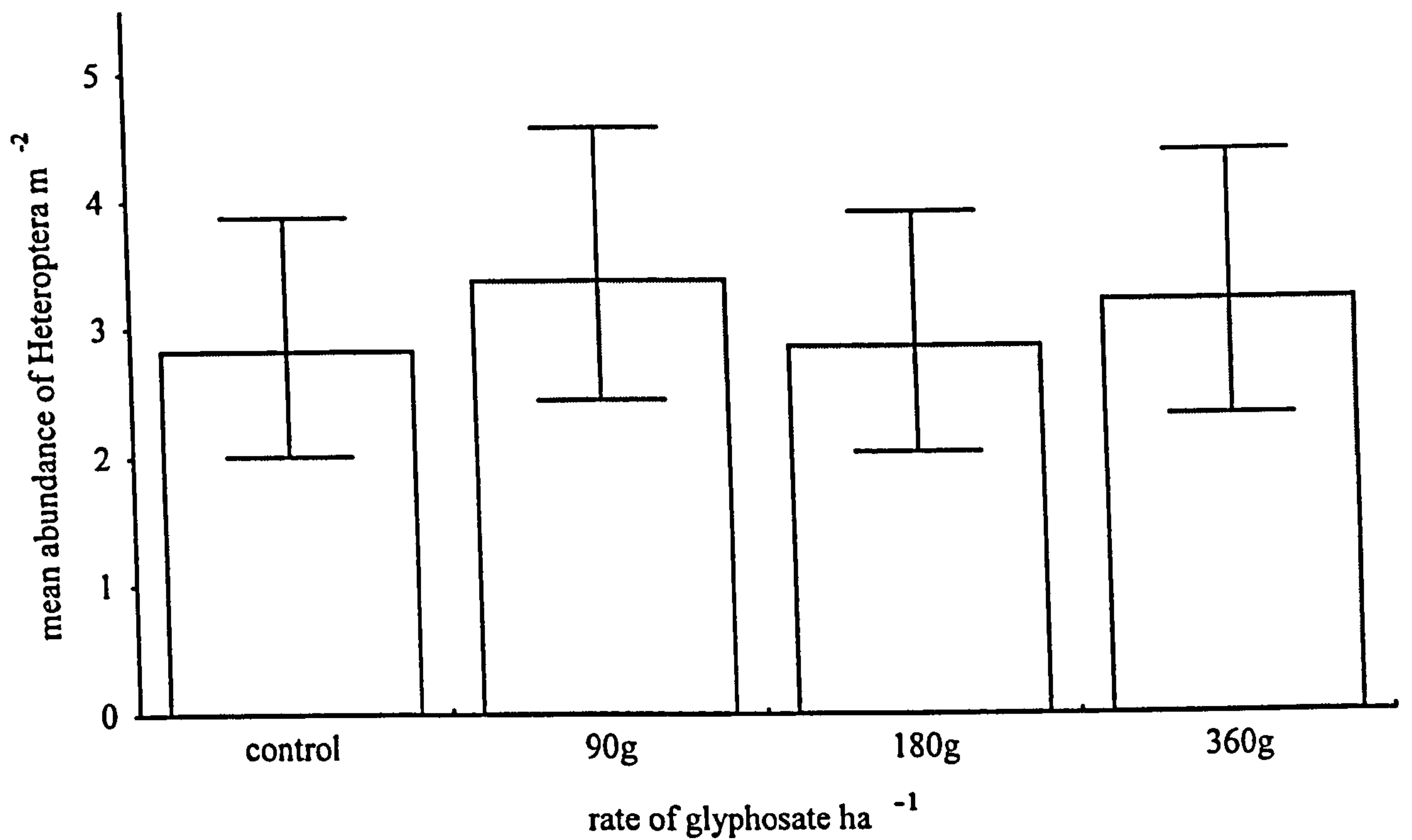


Figure 7.12. Back-transformed mean abundance of Heteroptera m⁻² and 95% confidence limits in the unsprayed control, 90g, 180g and 360g glyphosate ha⁻¹ treatments in 1997.

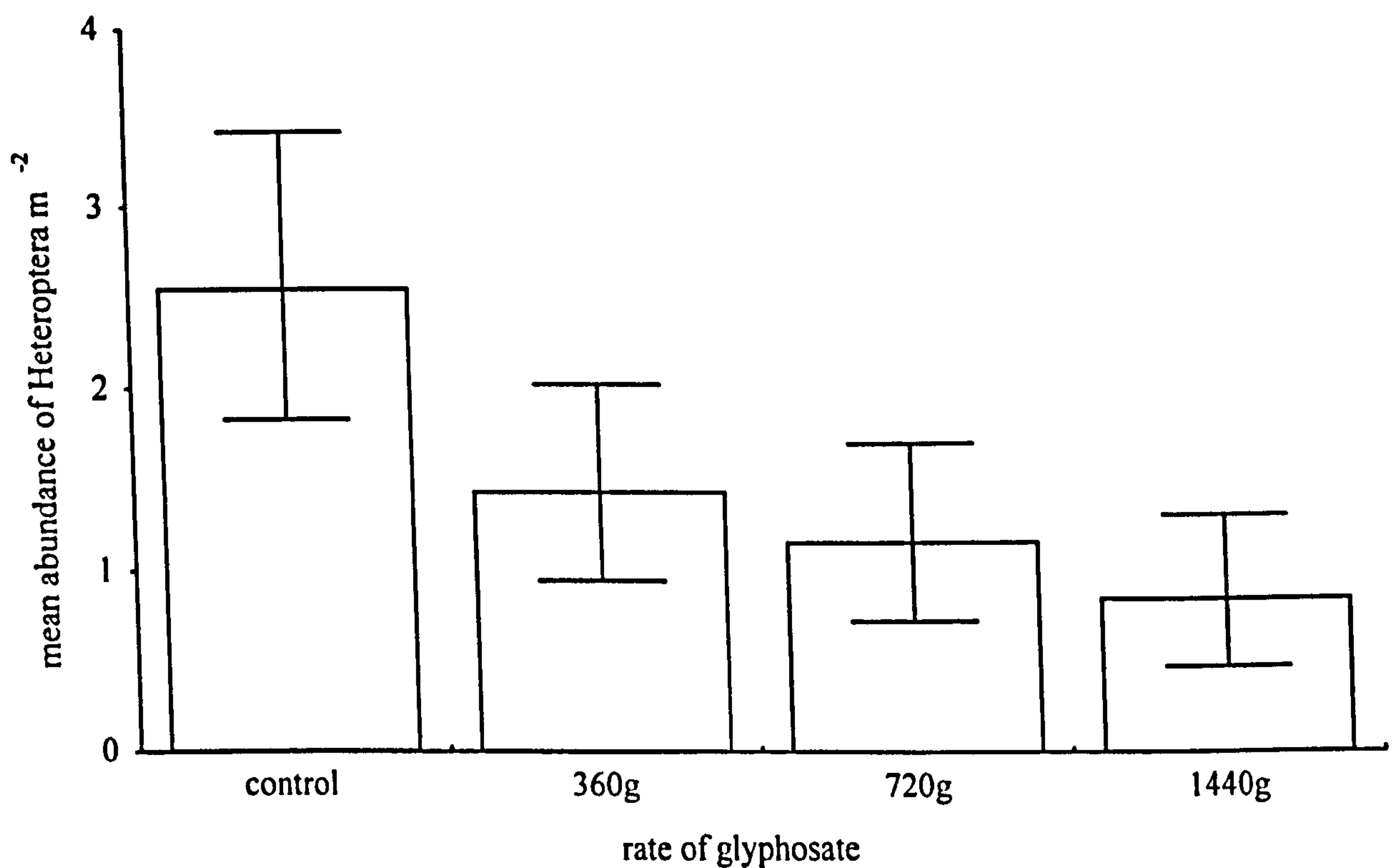


Figure 7.13. Back-transformed mean abundance of Heteroptera m⁻² and 95% confidence limits in the unsprayed control, 360g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

There was a significant positive relationship between vegetation height ($F_{(1, 158)} = 36.90$, $P < 0.001$, $r^2 = 0.19$) and abundance of Heteroptera and a significant negative relationship between amount of dead vegetation ($F_{(1, 158)} = 26.76$, $P < 0.001$, $r^2 = 0.14$) and abundance of Heteroptera from June to October when glyphosate was applied at less than 360g ha^{-1} (1997).

At the higher rates of 360g , 720g and 1440g glyphosate ha^{-1} (1998), there was a significant positive relationship between abundance of Heteroptera and vegetation height from June to October where vegetation height accounted for 38% of the variation in abundance ($F_{(1, 158)} = 98.90$, $P < 0.001$). There was also a significant negative relationship between abundance of Heteroptera and amount of dead vegetation from June to October under the higher rates, where amount of dead vegetation accounted for 13% of the variation in abundance ($F_{(1, 158)} = 24.65$, $P < 0.001$).

7.3.2.6 Phytophagous Heteroptera Abundance

Although 45 species of Heteroptera from 8 families were recorded from the experimental field margin plots over the two year sampling period (Appendix 4), no single species occurred in sufficient densities for analysis. Since the Heteroptera comprise phytophagous, predatory and omnivorous species, the abundance of Heteroptera in the treatments was analysed according to phytophagous and predatory feeding habit.

In 1997 (0g - 360g glyphosate ha^{-1} treatments) abundance of phytophagous Heteroptera significantly varied from June to October ($F_{(4, 12)} = 17.38$, $P < 0.001$) where abundance declined in August. There was also a significant date x treatment interaction indicating that the effect of treatment on abundance of phytophagous Heteroptera varied with

sampling date ($F_{(12, 112)} = 4.46, P<0.001$), therefore abundance was analysed for each sample date separately. The abundance of phytophagous Heteroptera varied significantly between treatments in June and July, but not in August, September or October (Table 7.16). In July, there were significantly fewer phytophagous Heteroptera in the glyphosate treated field margins than in the unsprayed control and in June, there were fewer in the 180g and 360g glyphosate ha⁻¹ treatments than in the 90g glyphosate ha⁻¹ treatments (Table 7.17). Figure 7.14 illustrates the back-transformed mean abundance of phytophagous Heteroptera m⁻² and 95% confidence limits in the 0g - 360g glyphosate ha⁻¹ treatments.

Table 7.16. One-way ANOVA results for abundance of phytophagous Heteroptera in unsprayed control (0g), 90g, 180g and 360g glyphosate ha⁻¹ treatments in June, July, August, September and October 1997.

	$F_{(3, 28)}$	P
June	3.21	0.038
July	11.12	0.001
August	2.84	0.056
September	0.67	0.577
October	1.09	0.368

Significant values at $P<0.05$ in bold.

Table 7.17. Contrast analysis results for differences in phytophagous Heteroptera abundance between unsprayed control (0g), 90g, 180g and 360g glyphosate ha⁻¹ treatments in 1997.

	June		July	
	$F_{(1, 28)}$	P	$F_{(1, 28)}$	P
0g v 90g	1.82	0.188	8.08	0.008
0g v 180g	1.94	0.175	11.40	0.002
0g v 360g	1.44	0.241	32.97	0.001
90g v 180g	7.51	0.011	0.29	0.597
90g v 360g	6.49	0.017	8.41	0.007
180g v 360g	0.04	0.848	5.60	0.025

Significant values at $P<0.05$ in bold.

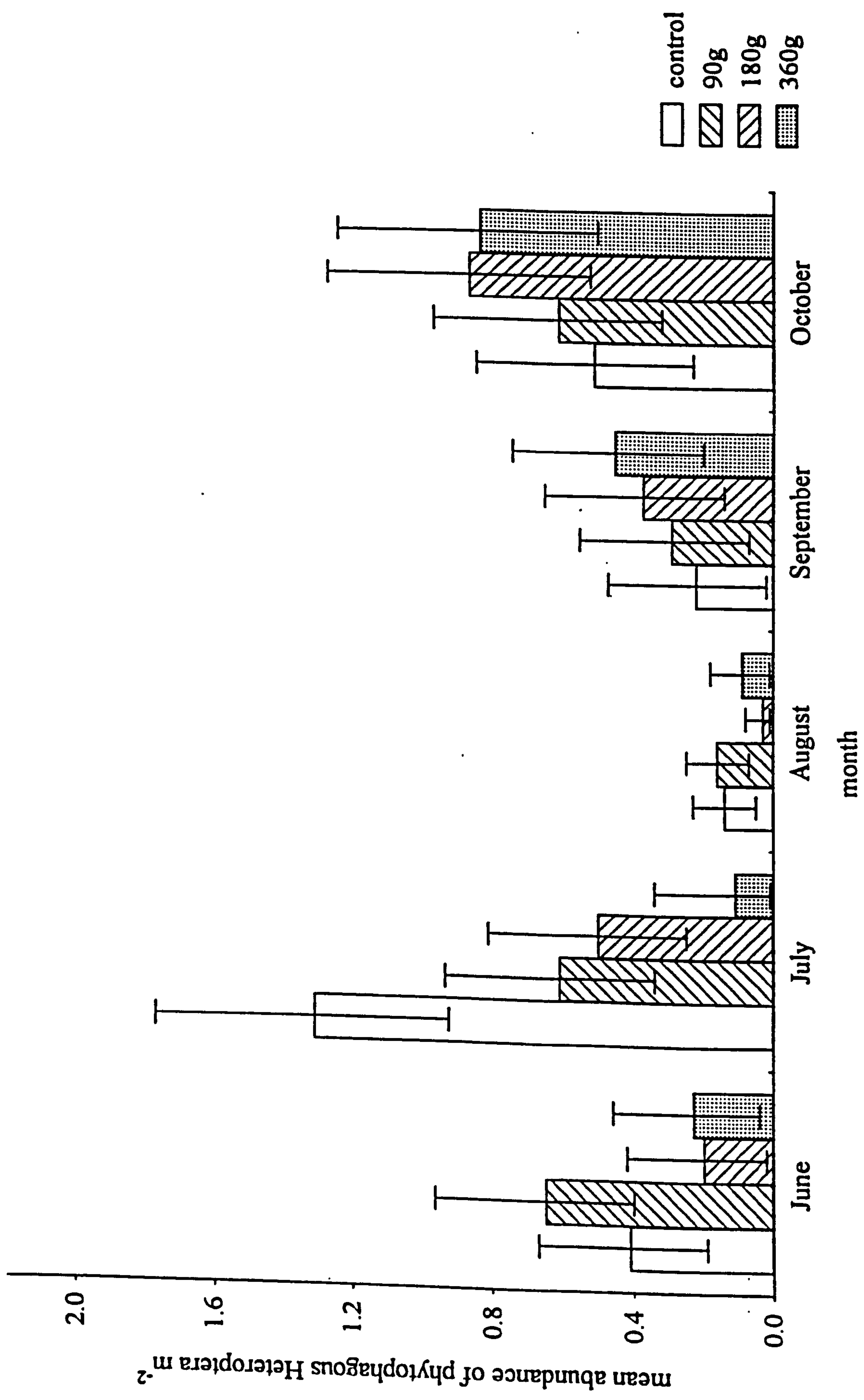


Figure 7.14. Back-transformed mean abundance of phytophagous Heteroptera m⁻² and 95% confidence limits in the unsprayed control, 90g, 180g and 360g glyphosate ha⁻¹ treatments in each sample month.

Abundance of phytophagous Heteroptera varied significantly from June to October in 1998 (0g - 1440g glyphosate ha⁻¹ treatments) ($F_{(4, 112)} = 2.99, P<0.05$), where abundance peaked in July. There was also a significant date x treatment interaction ($F_{(12, 112)} = 2.09, P<0.05$), therefore abundance was analysed for each month separately. The abundance of phytophagous Heteroptera varied significantly between the 0g -1440g glyphosate ha⁻¹ treatments in July, August, September and October (Table 7.18), where abundance was significantly greater in the unsprayed control than in all other treatments (Table 7.19). Figure 7.15 illustrates the back-transformed mean abundance of phytophagous Heteroptera m⁻² and 95% confidence limits in the 0g - 1440g glyphosate ha⁻¹ treatments.

Table 7.18. One-way ANOVA results for abundance of phytophagous Heteroptera in unsprayed control (0g), 360g, 720g and 1440g glyphosate ha⁻¹ treatments in June, July, August, September and October 1998.

	$F_{(3, 28)}$	P
June	2.51	0.079
July	16.56	0.001
August	8.01	0.001
September	22.24	0.001
October	11.26	0.001

Significant values at $P<0.05$ in bold.

Table 7.19. Contrast analysis results for differences in phytophagous Heteroptera abundance between the unsprayed control (0g), 360g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

	July		August		September		October	
	$F_{(1, 28)}$	P	$F_{(1, 28)}$	P	$F_{(1, 28)}$	P	$F_{(1, 28)}$	P
0g v 360g	25.01	0.001	14.60	0.001	44.49	0.001	15.00	0.001
0g v 720g	34.49	0.001	16.67	0.001	44.49	0.001	211.12	0.001
0g v 1440g	37.72	0.001	16.37	0.001	44.49	0.001	28.30	0.001
360g v 720g	0.76	0.391	0.07	0.795	0.01	0.999	0.52	0.476
360g v 1440g	1.30	0.264	0.07	0.795	0.01	0.999	2.09	0.159
720g v 1440g	0.072	0.790	0.01	0.999	0.01	0.999	0.52	0.476

Significant values at $P<0.05$ in bold.

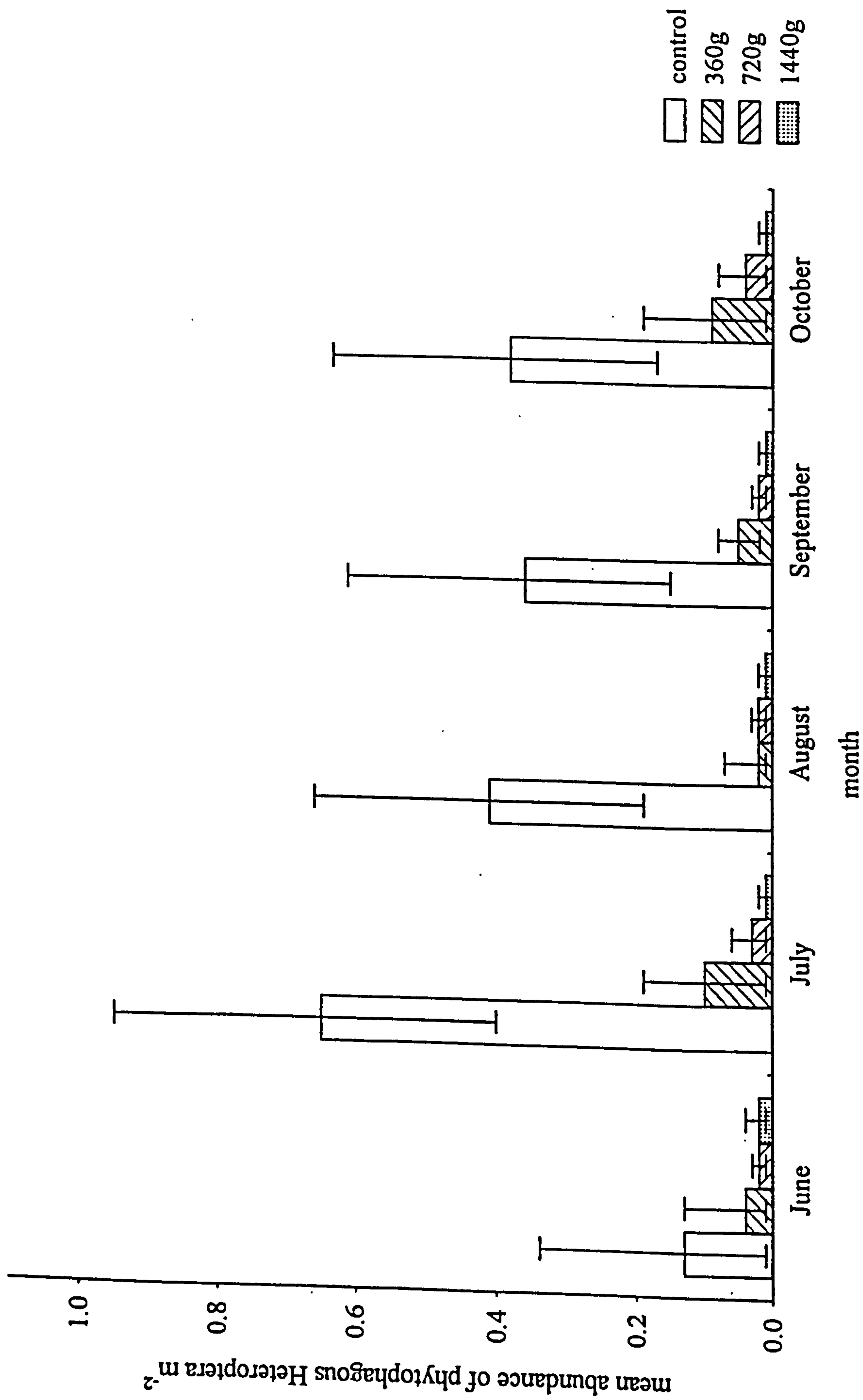


Figure 7.15. Back-transformed mean abundance of phytophagous Heteroptera m⁻² and 95% confidence limits in the unsprayed control, 360g, 720g and 1440g glyphosate ha⁻¹ treatments in each sample month.

When glyphosate was applied at less than 360g ha^{-1} (1997) there was a significant positive relationship between abundance of phytophagous Heteroptera and vegetation height in July, where vegetation height accounted for 25% of the variation in abundance. There was a significant negative relationship between abundance of phytophagous Heteroptera and amount of dead vegetation in July, where amount of dead vegetation accounted for 35% of the variation in abundance (Table 7.20).

At the higher rates of 360g , 720g and 1440g glyphosate ha^{-1} (1998), there was a significant positive relationship between abundance of phytophagous Heteroptera and vegetation height in June, July, August and September, where vegetation height accounted for up to 44% of the variation in abundance. There was also a significant negative relationship between abundance of phytophagous Heteroptera and amount of dead vegetation in June, July, August and September under the higher rates where amount of dead vegetation accounted for up to 63% of the variation in abundance (Table 7.20).

Table 7.9. Regression analysis of the effect of vegetation height (Height) and amount of dead vegetation (Dead) on the abundance of Araneae m⁻² in 0g - 360g glyphosate ha⁻¹ (1997) and 0g - 1440g glyphosate ha⁻¹ (1998) treatments.

	June				July				August				September				October			
	a	b	r ²	P	a	b	r ²	P	a	b	r ²	P	a	b	r ²	P	a	b	r ²	P
Height																				
1997	2.65	-0.01	0.08	0.114	2.54	-0.01	0.06	0.195	2.94	-0.01	0.01	0.996	3.24	0.01	0.11	0.059	4.09	-0.01	0.01	0.652
1998	2.11	-0.01	0.01	0.938	2.02	0.01	0.14	0.032	2.80	0.01	0.06	0.186	3.09	0.01	0.15	0.029	3.01	0.02	0.53	0.001
Dead																				
1997	2.37	-0.02	0.03	0.323	2.24	0.01	0.06	0.195	2.95	-0.01	0.01	0.813	3.68	-0.01	0.03	0.306	3.98	0.01	0.04	0.284
1998	2.10	0.01	0.01	0.899	2.44	-0.01	0.25	0.003	3.11	-0.01	0.04	0.276	3.51	-0.01	0.19	0.014	3.61	-0.01	0.24	0.005

Significant values at $P<0.05$ in bold.

7.3.2.7 Predatory Heteroptera Abundance

The abundance of predatory Heteroptera varied significantly from June to October in 1997 (0g - 360g glyphosate ha⁻¹) ($F_{(4, 112)} = 120.20, P < 0.001$) and in 1998 (0g - 1440g glyphosate ha⁻¹) ($F_{(4, 112)} = 130.51, P < 0.001$), where abundance decreased through the season. There were no significant date x treatment interactions in either 1997 ($F_{(12, 112)} = 1.31, P > 0.05$) or 1998 ($F_{(12, 112)} = 0.92, P > 0.05$). However, due to a significant departure from sphericity within repeated measures ANOVA of abundance of predatory Heteroptera in the 0g - 360g glyphosate ha⁻¹ treatments and in the 0g - 1440g glyphosate ha⁻¹ treatments (Mauchly's $W = 0.47, P < 0.05$; Mauchly's $W = 0.22, P < 0.001$, respectively) a multivariate approach to repeated measures was computed for the data from both years.

Abundance of predatory Heteroptera did not significantly vary between the 0g - 360g glyphosate ha⁻¹ treatments ($R_{(15, 66)} = 1.01, P > 0.05$) or between the 0g - 1440g glyphosate ha⁻¹ treatments ($R_{(15, 66)} = 1.34, P > 0.05$). Figures 7.16 and 7.17 illustrate the back-transformed mean abundance of predatory Heteroptera m⁻² and 95% confidence limits in the 0g - 360g and 0g - 1440g glyphosate ha⁻¹ treatments respectively.

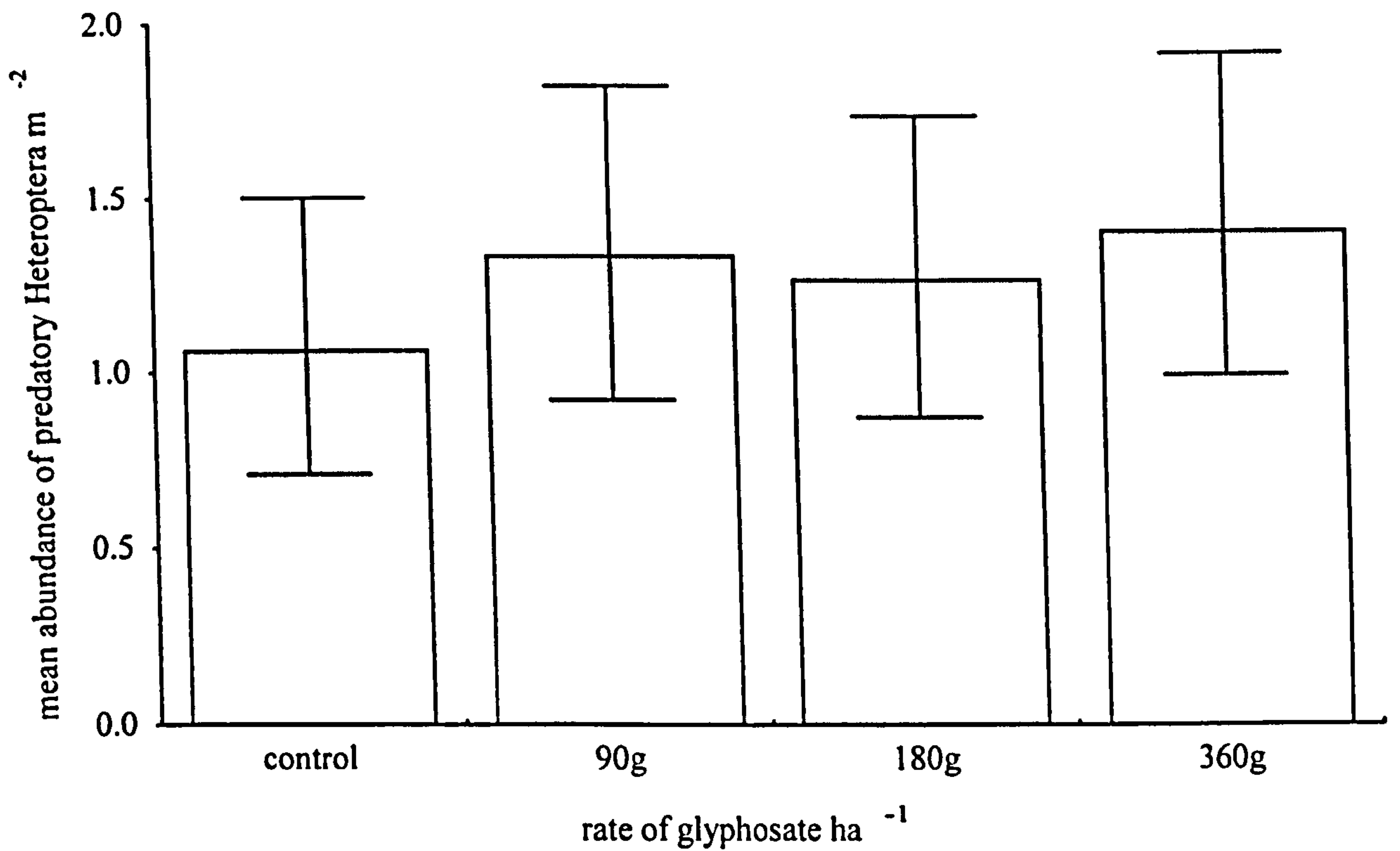


Figure 7.16. Back-transformed mean abundance of predatory Heteroptera m⁻² and 95% confidence limits in the unsprayed control, 90g, 180g and 360g glyphosate ha⁻¹ treatments in 1997.

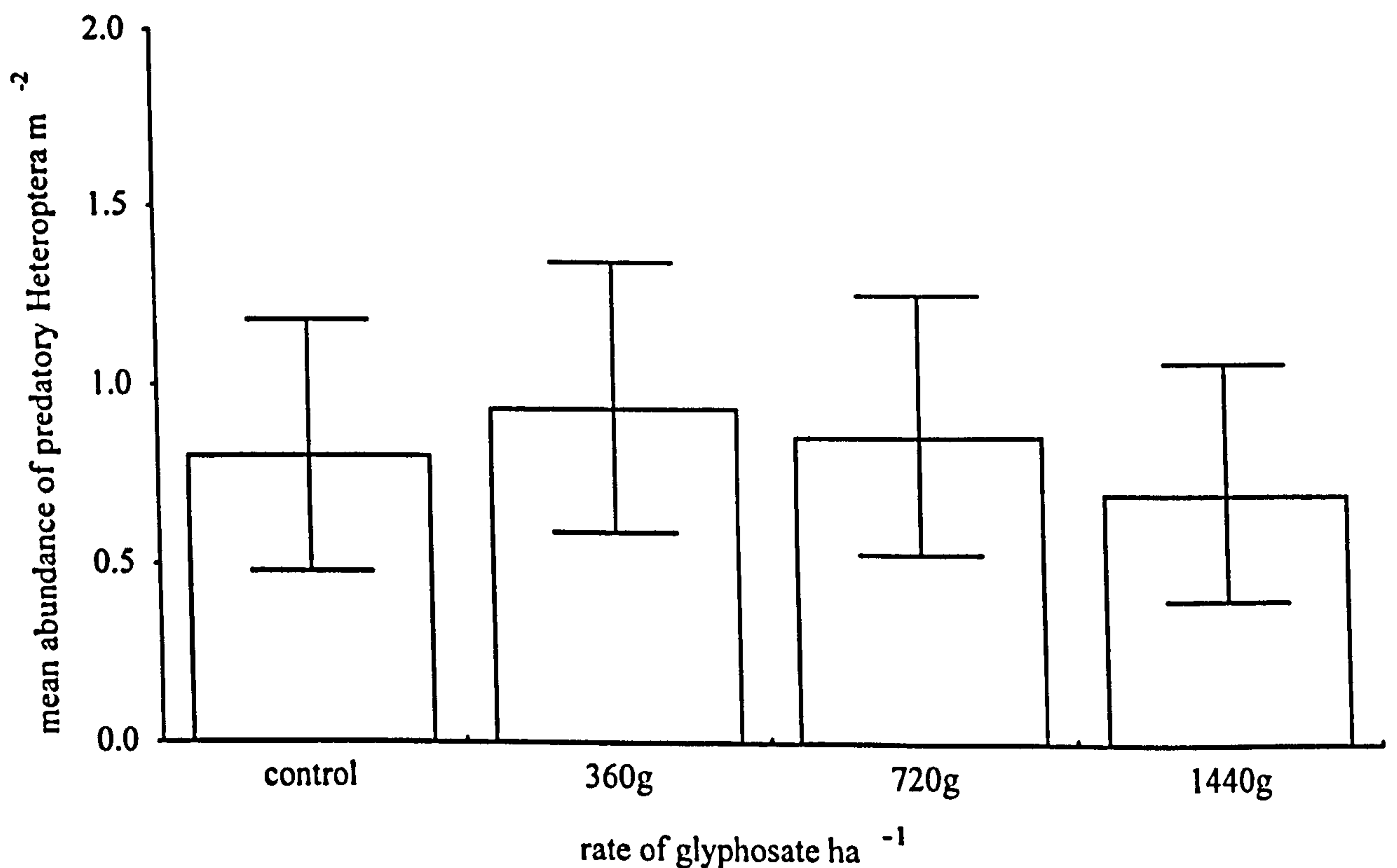


Figure 7.17. Back-transformed mean abundance of predatory Heteroptera m⁻² and 95% confidence limits in the unsprayed control, 360g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

When glyphosate was applied at less than 360g ha⁻¹ (1997), there was a significant positive relationship between abundance of predatory Heteroptera and vegetation height ($F_{(1, 158)} = 19.49$, $P < 0.001$, $r^2 = 0.11$). There was also a significant negative relationship between abundance of predatory Heteroptera and amount of dead vegetation ($F_{(1, 158)} = 16.42$, $P < 0.001$, $r^2 = 0.09$).

At the higher rates of up to 1440g glyphosate ha⁻¹ (1998), there was a significant positive relationship between abundance of predatory Heteroptera and vegetation height ($F_{(1, 158)} = 26.48$, $P < 0.001$, $r^2 = 0.14$), but in contrast to 1997, there was no significant relationship between abundance of predatory Heteroptera and amount of dead vegetation ($F_{(1, 158)} = 2.89$, $P > 0.05$).

7.3.3 Community Analyses

Araneae, Carabidae and Heteroptera species abundance data from the 0g - 360g glyphosate ha⁻¹ treatments and from the 0g - 1440g glyphosate ha⁻¹ treatments were ordinated by DECORANA (Hill, 1994).

The Araneae, Carabidae and Heteroptera communities in the 0g - 360g glyphosate ha⁻¹ (1997) treatments did not show any distinct separation along either Axis 1 or Axis 2, where the main cluster comprised communities from all treatments. However, two communities from the 90g glyphosate ha⁻¹ treatment and one each from the unsprayed control and 180g glyphosate ha⁻¹ treatments were isolated from the main cluster as satellites. Communities from four of the field margin plots treated with 360g glyphosate ha⁻¹ were separated from the main cluster and appeared to be separated along Axis 1. Axis 1 was negatively

correlated with vegetation height ($r = -0.35$, $P < 0.05$) indicating that communities with higher Axis 1 scores were associated with shorter vegetation (Figure 7.18).

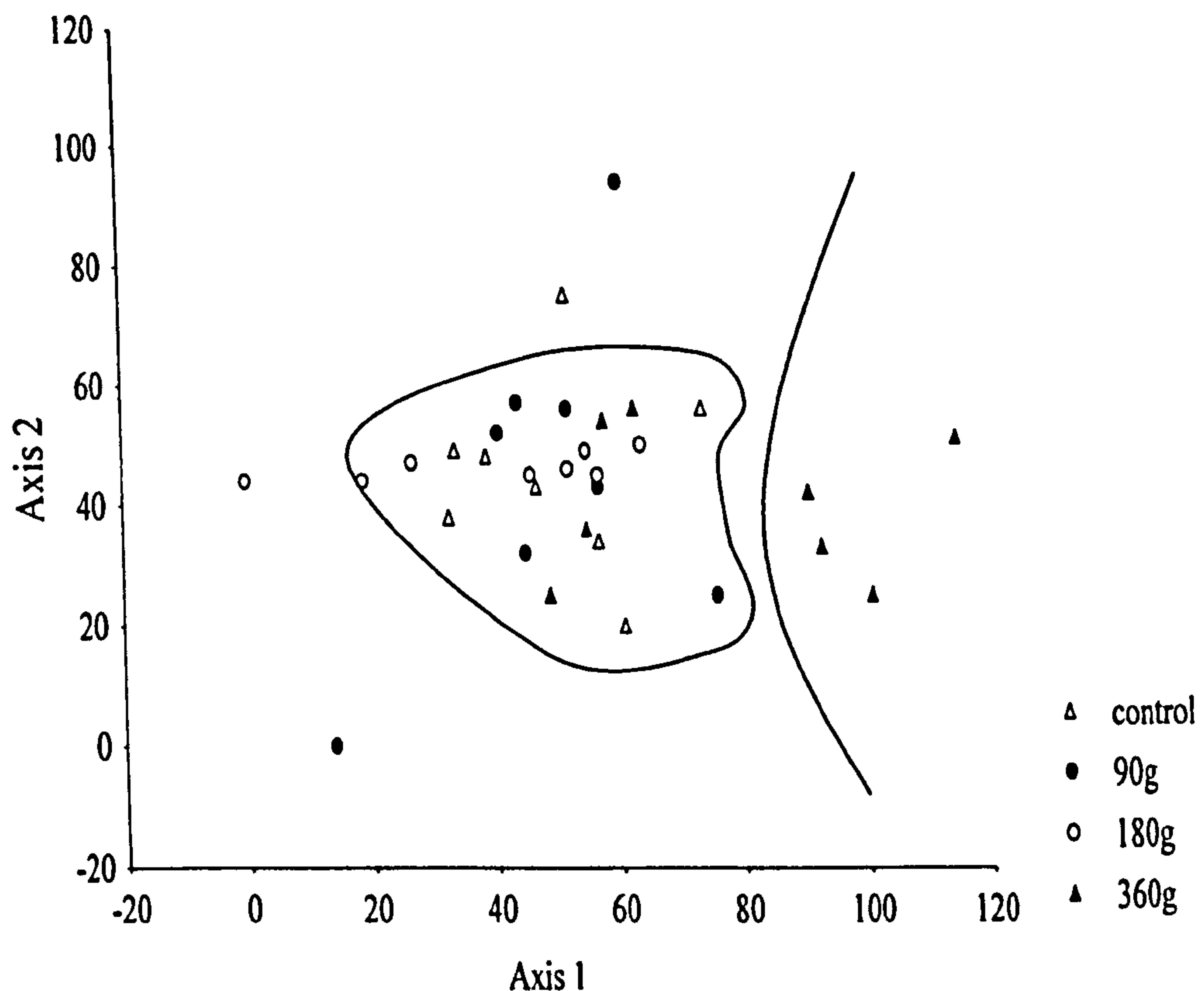


Figure 7.18. Axis 1 by axis 2 plots of DCA ordination scores for the Araneae, Carabidae and Heteroptera community in the unsprayed control, 90g, 180g and 360g glyphosate ha^{-1} treatments in 1997.

In 1998, the Araneae, Carabidae and Heteroptera communities in the unsprayed control were clearly separated from the main cluster along Axis 1. The main cluster comprised communities from the 360g, 720g and 1440g glyphosate ha^{-1} treatments, however, there were three satellite communities in the ordination: two from the 1440g and one from the 720g glyphosate ha^{-1} treatments (Figure 7.19). Axis 1 was negatively correlated with vegetation height and positively correlated with amount of dead vegetation and rate of glyphosate, indicating that communities with higher Axis 1 scores were associated with

shorter vegetation, greater coverage by dead vegetation and higher rates of glyphosate (Table 7.21).

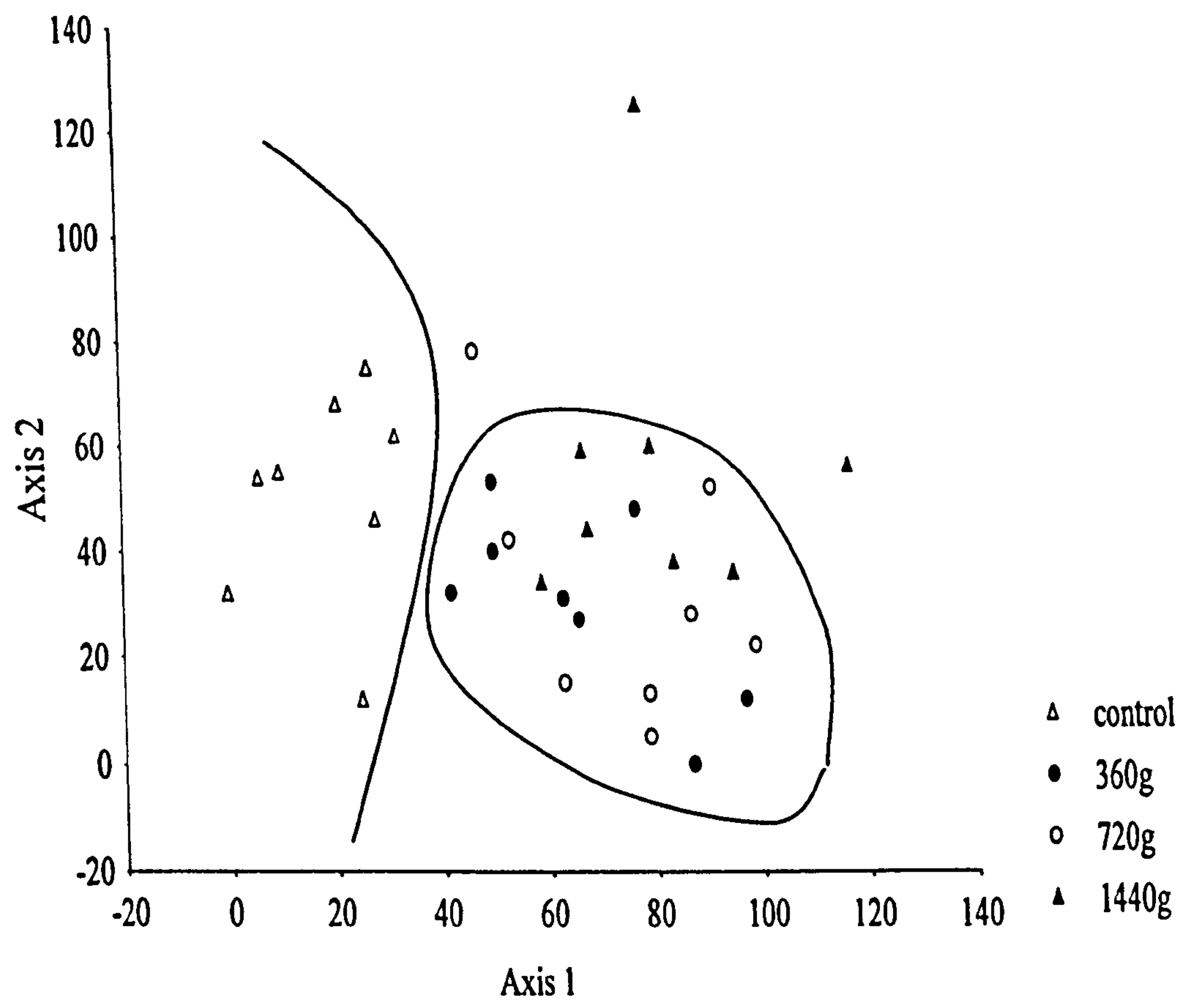


Figure 7.19. Axis 1 by axis 2 plots of DCA ordination scores for the Araneae, Carabidae and Heteroptera community in unsprayed control, 360g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

Table 7.21. Spearman rank correlation between Axis 1 scores and vegetation height, dead vegetation cover and rate of glyphosate for the Araneae, Carabidae and Heteroptera communities in the unsprayed control (0g), 360g, 720g and 1440g glyphosate ha⁻¹ treatments in 1998.

	Spearman's R	<i>P</i>
vegetation height	-0.73	0.001
dead vegetation cover	0.74	0.001
rate of glyphosate	0.71	0.001

Significant values at *P*<0.05 in bold.

7.3.4 Arthropod Abundance at 12 and 16 Months After Glyphosate Application

By 12 and 16 months after the application of glyphosate there was no longer any dead vegetation present in the field margin plots and there was no association between rate of glyphosate and vegetation height ($r^2 = -0.35 = P>0.05$; $r^2 = -0.24, P>0.05$, at 12 and 16 months after glyphosate application respectively).

There were no significant differences in abundance of Araneae, Carabidae or Heteroptera between the unsprayed control and 360g glyphosate ha⁻¹ treatment at either 12 months or 16 months after the application of the glyphosate (Table 7.22).

Table 7.22. ANOVA results of abundance of Araneae, Carabidae and Heteroptera in the unsprayed control (0g) and 360g glyphosate ha⁻¹ treatments at 12 (May) and 16 (September) months after the application of the glyphosate.

		May 1998				September 1998			
		Mean	SE	$F_{(1, 14)}$	P	Mean	SE	$F_{(1, 14)}$	P
Araneae	0g	70.25	4.16	0.01	0.976	190.50	23.56	1.33	0.268
	360g	70.00	6.93			159.88	12.30		
Carabidae	0g	3.13	0.81	0.43	0.523	15.00	2.87	0.04	0.841
	360g	2.50	0.50			14.25	2.28		
Heteroptera	0g	24.13	4.41	0.01	0.968	4.13	0.67	0.89	0.363
	360g	24.38	4.15			5.38	1.15		

Three species of spider occurred in sufficient abundance for further analysis. By 12 months after the glyphosate application, abundance of *Lepthyphantes ericaeus* was significantly greater in the unsprayed control than in the 360g glyphosate ha⁻¹ treatments, but abundance of *P. juncea* was not significantly different between the two treatments.

The abundance of *L. ericaeus* was not significantly related to vegetation height ($F_{(1, 14)} =$

0.28, $P>0.05$). By 16 months after the glyphosate application, the abundance of *L. tenuis*, *L. ericaeus* or *P. degeeri* was not significantly different between the two treatments (Table 7.23).

Table 7.23. ANOVA results of abundance of spider species in the unsprayed control (0g) and 360g glyphosate ha⁻¹ treatments at 12 (May) and 16 (September) months after the application of the glyphosate.

		Mean	SE	$F_{(1, 14)}$	P
May					
<i>L. ericaeus</i>	0g	0.52	0.08	6.52	0.023
	360g	0.21	0.05		
<i>P. juncea</i>	0g	2.00	0.22	3.57	0.080
	360g	3.53	0.08		
September					
<i>L. tenuis</i>	0g	1.34	0.32	0.38	0.548
	360g	1.18	0.11		
<i>L. ericaeus</i>	0g	0.57	0.13	0.07	0.801
	360g	0.52	0.09		
<i>P. degeeri</i>	0g	0.42	0.12	0.40	0.535
	360g	0.27	0.14		

Significant values at $P<0.05$ in bold.

No single species of Heteroptera occurred in sufficient densities for further analysis, therefore the abundance of two feeding guilds in the unsprayed control and 360g glyphosate ha⁻¹ treatments was analysed. There was no significant difference in abundance of either phytophagous or predatory Heteroptera by 12 or 16 months after the application of glyphosate (Table 7.24).

Table 7.24. ANOVA results of abundance of phytophagous and predatory Heteroptera in the unsprayed control (0g) and 360g glyphosate ha⁻¹ treatments at 12 (May) and 16 (September) months after the application of the glyphosate.

		Mean	SE	$F_{(1, 14)}$	P
May					
Phytophagous	0g	11.00	3.85	0.21	0.888
	360g	10.38	2.01		
Predatory	0g	11.50	1.50	0.32	0.583
	360g	13.50	3.22		
September					
Phytophagous	0g	1.63	0.46	1.06	0.321
	360g	2.38	0.56		
Predatory	0g	1.63	0.42	1.58	0.230
	360g	1.00	0.27		

No species of Carabidae occurred in sufficient densities, therefore further analysis was not possible.

7.4 Discussion

The experimental plot size was small for this type of study. However, having taken practical constraints into consideration, it was decided that it would be better to use the longest length of field margin with the same aspect and adjacent to one crop type rather than extend plot size and therefore be required to use plots with different aspects (and degrees of insolation) and possibly crop type. It was thought that the benefits of maintaining greater randomised block replication of the treatments outweighed those of longer plot sizes. The effects of the treatments in this experiment were analysed relative to each other, and it was assumed that the chances of arthropods occupying a certain area

should be independent of plot length, since other obvious non-treatment variables were equal, i.e. proximity and type of hedge and other sources of migration, adjacent crop type and crop husbandry. Furthermore, the herbicide was directly affecting the habitat, rather than the arthropods, thus abundance of individuals was a reflection of habitat suitability.

Discussion of the results of this experiment will focus on the arthropods, with an overview of the effects of rates of glyphosate on the vegetation. The influence of the effects of changes in vegetation height and cover of dead vegetation will be discussed with the arthropod groups where appropriate.

7.4.1 Vegetation

Increasing the rate of glyphosate (0g – 360g and 0g – 1440g glyphosate ha⁻¹) was associated with reductions in vegetation height and increases in the amount of cover by dead vegetation. These effects of increasing rate of glyphosate (apart from rates less than 360g ha⁻¹) were detectable from two weeks after spray application until the end of the sampling programme in October. The effect of rates of glyphosate less than 360g ha⁻¹ on cover of dead vegetation were detectable 6 weeks after spray application (July) and were not apparent by 14 weeks after application (September). Therefore, at the lower rates of glyphosate, vegetation health was able to recover by 14 weeks after spray application, but vegetation height remained reduced. Recovery, but the lack of increase in vegetation height was due to tillering of grasses within the field margin plots.

7.4.2 Arthropods

7.4.2.1 Sampling Methodology

Suction sampling is a long established method of sampling epigeal arthropods in grassland and arable crops (e.g., Dietrick, 1961; Southwood, 1980; Thornhill, 1978), but the earlier sampling devices (Dietrick-vac and Thornhill vacuum sampler) are expensive, large, heavy and cumbersome to use, usually requiring more than one person to operate the machine in the field.

Recently, the efficiency of hand-held, modified machines designed for collecting leaf-litter and other light-weight garden material garden (garden-vacs) has been assessed for epigeal arthropod sampling in grassland and arable crop situations. Sampling epigeal arthropods is usually achieved using one sampling method, due largely to labour costs and available time, however, sampling efficiency is usually compromised. For example, the traditional suction samplers under-sample larger arthropods, such as the larger Carabidae and Lycosidae (Mommertz *et al.*, 1996), while pitfall trapping, which is used for sampling carabids and cursorial spiders (e.g. Lycosidae), is a reflection of behaviour, activity (Greenslade, 1964) and strata at which the species are active (Topping & Sunderland, 1992). Garden-vacs, however, have been shown to be more efficient than the traditional suction samplers in sampling the larger Carabidae and therefore represent a better option for achieving more robust sampling programmes. Due to the reduced aperture of the garden-vac's collection tube, mean air-flow of the garden-vacs is faster than that of the traditional suction samplers (MacLeod *et al.*, 1995; Stewart & Wright, 1995). The increased airflow results in an increased sampling efficiency of species that inhabit the litter and soil surface, including the linyphiid spiders (Stewart & Wright, 1995).

It is important, though, to recognise the limitations of the garden-vac used in this experiment, which was also the model investigated by MacLeod *et al.* (1994; 1995). Although larger, heavier and more mobile arthropods, i.e. cursorial spiders, larger Carabidae and Staphylinidae, are more efficiently sampled with a garden-vac when compared with a D-vac, they nevertheless remain under-sampled (Sunderland, pers. comm.) and edge effect of the increased surface area of the aperture of the garden-vac's collecting tube compared with that of the traditional suction sampler can lead to enhanced catch sizes (Samu *et al.*, 1997). Therefore, it is stressed that the analyses of arthropod abundance data from this experiment are relative to each other and are only considered in this context.

7.4.2.2 Araneae

The Araneae were the most abundant of the three arthropod groups studied and were dominated by the web-spinning Linyphiidae (Appendix 2), while relatively few representatives of cursorial species were found.

The abundance of Araneae (spiders) in the experimental field margin plots was reduced by applications of different rates of glyphosate. In September 1997, glyphosate applied at 360g ha⁻¹ resulted in a significantly lower abundance of spiders than in each of the other glyphosate treated plots and the unsprayed control plots. When glyphosate application rates were greater than 360g ha⁻¹ (1998), effects on spider abundance were more rapid and prolonged, since effects were detected earlier and continued to be significant throughout the remainder of the sampling season. Indeed, the effects of the 1440g glyphosate ha⁻¹ treatment were detectable from July to October, while the effects of the 720g glyphosate ha⁻¹ treatment became apparent later in the season, in September and October. Effects of

the lower rate of 360g glyphosate ha⁻¹ became apparent in October, but were also unexpectedly detected earlier in the season in July. The reason for the significant difference in abundance of spiders between the 360g glyphosate ha⁻¹ treatment and the unsprayed control in July is not clear, especially since there were no similar results for the higher rates of glyphosate in the following year. In each case of significant treatment effects, there were greater abundances of spiders in the unsprayed plots than in the sprayed plots. Therefore, glyphosate applied at rates of 360g ha⁻¹ and more significantly reduced the abundance of spiders in the experimental grassy arable field margin plots.

Abundance of spiders was not only different between the treated and untreated plots, but also between the rates of glyphosate. For example, in 1997, there were more spiders m⁻² in the 90g and 180g glyphosate ha⁻¹ treatments than in the 360g glyphosate ha⁻¹ treatment, while in 1998, there were more spiders in the 360g glyphosate ha⁻¹ treatment than in the 1440g glyphosate ha⁻¹ treatment. These results suggest that increasing the rate of glyphosate can continue to reduce the abundance of spiders. Although this suggests a positive relationship between rate of glyphosate and spider abundance, it was not possible to combine the two years' data sets for regression analysis since the abundance of spiders in the experimental field margins in 1998 were significantly lower than in the field margin used in 1997 (Table 7.4).

Similar results of effects of herbicide applications on Araneae in crop headlands and non-cropped areas have also been recorded. In perhaps the earliest experiment studying the effects of herbicide applications to cereals on spiders, Raatikainen & Huhta (1968) found that there were fewer spiders in MCPA-treated oats than in untreated oats and established the importance of weediness in crops to spiders. Weed control was also cited as the cause of reduction in spiders in cotton crops in the US (Stam *et al.*, 1978) and in the headlands of

winter wheat (Moreby & Southway, 1999). In a study of the effects of annual June applications of glyphosate (1080g ha^{-1}), Baines *et al.* (1998) found that abundance of Araneae was reduced, however, effect on species richness was not significant.

Baines *et al.* (1998) suggested that it was the collapse in plant stems, and hence reduction in vegetation height, that contributed to the decrease in spider abundance in grassy field margins treated with glyphosate. In this experiment, increasing the rate of glyphosate generally led to shorter vegetation and increased cover by dead vegetation for the duration of the sampling season. The exception was at the lower rates of glyphosate, where the relationship between rate of glyphosate and cover by dead vegetation was detectable in July and August only. Reductions in the abundance of spiders were positively related to vegetation height and negatively related to the cover of dead vegetation. The relationship between Araneae abundance and vegetation height, however, was stronger than for cover by dead vegetation when glyphosate was applied at more than 360g ha^{-1} (Table 7.9), suggesting that vegetation height was the more important factor in determining spider abundance. Despite there being no significant relationships between vegetation height or cover of dead vegetation with abundance of spiders at rates less than 360g ha^{-1} in September when spider abundance varied between treatments, the relationship with vegetation height was only just non-significant ($P = 0.059$; Table 7.9), thus height may be important in determining the distribution of spiders at the lower rates of glyphosate application.

By September, field margin vegetation had begun to recover, since there was new plant growth (indicated by a reduction in amount of dead vegetation), but there had not been sufficient growth to increase the vegetation height to that comparable with the height in unsprayed plots. Furthermore, the fact that there were associations between rate of

glyphosate and vegetation height in all months, but only a significant difference in spider abundance in September only, suggests there may have been an increase in a particular group of spiders (general abundance increased in September and October (Figure 7.5)), for whom vegetation height is important. The lack of differences in abundance of spiders in October may be explained by the reduction in strength of association between rate of glyphosate and vegetation height and/or the reduced presence of a group of spiders strongly dependent on taller vegetation.

Herbicide, by its very nature, kills plants and therefore reduces the associated variation in height structure and diversity of plant architecture. Changes in plant structural complexity were correlated with a reduction in cursorial spiders in cereal headland (White & Hassall, 1994). Vegetation height, which is an important component of habitat microspatial heterogeneity, has been shown to be a good indicator of structural diversity (Brown, 1991). Habitat microspatial heterogeneity theory dictates that the more structurally heterogeneous a habitat is, the more diverse the communities will be (MacArthur & MacArthur, 1961). The influence of vegetation height on spider abundance and diversity is well documented (e.g., Greenstone, 1984; de Keer *et al.*, 1989; Uetz, 1991; Wise, 1993), and is one of the most important determinants of a local spider fauna, particularly for the web-spinning species. As vegetation structural diversity becomes more complex, not only does the microhabitat become more stable (de Keer *et al.*, 1989), but opportunities for web site selection, and therefore prey capture, increase (Wise, 1993). Gibson *et al.* (1992a) also found that spider assemblages in grazed grassland were affected more by plant architecture than plant species composition. In this experiment, decreasing vegetation height was related to increasing rates of glyphosate, and changes in microhabitat quality associated with shorter vegetation (decreased humidity, increased insolation, reduction of web-site availability, reduced diversity in plant architecture) are thought to have led to the decrease in

abundance of the spider fauna.

There were two species of spider in this experiment that occurred in sufficiently high numbers for further analysis: *L. tenuis* & *G. rubens* and are both web-spinners from the Linyphiidae.

When compared with the unsprayed experimental field margin plots, abundance of *L. tenuis* was significantly reduced by glyphosate applied at rates of 360g ha⁻¹ and more. Furthermore, there were significant differences in abundance between the glyphosate treatments, where there were more spiders m⁻² in the 360g glyphosate ha⁻¹ treatment than in the 720g and 1440g glyphosate ha⁻¹ treatments (Table 7.12). This suggests that although *L. tenuis* continues to be present where glyphosate is applied at rates of greater than 720g glyphosate ha⁻¹, abundance may not be further significantly reduced. Replication of this experiment over more than one season would confirm whether 720g glyphosate ha⁻¹ represented a threshold-rate for *L. tenuis*.

In contrast to total abundance of spiders, the abundance of *L. tenuis* was not related to vegetation height, however, it was negatively related to amount of dead vegetation, although the relationship in both years was low (both 9%). This is a curious result, especially considering the results of intensive autecological studies of *L. tenuis* (e.g. Alderweireldt, 1989; 1994). In investigating the prey capture strategies of *L. tenuis*, Alderweireldt (1994) found that the species was not flexible in its choice of web placement as it always used vegetation structures for web attachment, and usually constructed webs at around 10cm above ground level. In this experiment, vegetation height was reduced by increasing rates of glyphosate, and it was expected that where vegetation height was reduced to little more than 10cm (Figure 7.2), this would have influenced the abundance of

L. tenuis. It may be possible that *L. tenuis* can withstand reductions in vegetation height, as long as height is not less than the required 10cm for web-building. For example, the species is well adapted to areas with low vegetation cover and little litter with moderate disturbance (Rushton *et al.*, 1987; Alderweireldt, 1989).

Nevertheless, abundance of *L. tenuis* was significantly reduced by glyphosate applications, and the effects were indirect, since glyphosate is not toxic (Chapter 6) and by having excellent dispersal capabilities, this species is able to rapidly and easily exploit more appropriate habitat (Topping & Sunderland, 1998). Suitable micro-climate is known to be an important factors in web site selection for *L. tenuis* (Samu *et. al.*, 1996). Although not measured in this experiment, it is believed that humidity levels decreased with the decrease in vegetation height and amount of lush vegetation. However, these factors are correlated with changes in vegetation height (de Keer *et al.*, 1989) and vegetation height did not influence the abundance of *L. tenuis*. Therefore, the effect of glyphosate on this species may be more complex and related to prey availability.

Topping & Sunderland (1998) suggest that dispersal in *L. tenuis* may be prompted by the avoidance of adverse conditions and Samu *et al.*, (1996) suggest that food deprivation may be a cause of web abandonment by *L. tenuis*. Starvation-induced dispersal has been observed in other linyphiid spiders (Weyman *et al.*, 1994), and this may be the case for *L. tenuis*. Aphididae constituted more than 80% of the diet of *L. tenuis* (Alderweireldt, 1994) and since in this experiment phytophagous Heteroptera were reduced in abundance by glyphosate applications, it is likely than aphids also suffered similar reductions. Aphids require healthy plant material to feed from (Dolling, 1991), and, cover by healthy vegetation decreased with increasing amount of glyphosate in the experimental plots. Abundance of *L. tenuis* was also weakly negatively related to cover by dead vegetation,

therefore it is possible that a reduced prey availability, caused by an impoverished food source contributed to decline in abundance of *L. tenuis*, although the precise causes cannot be specified here.

G. rubens was even more sensitive to applications of glyphosate than *L. tenuis*, since it was significantly reduced by lower rates (180g and 360g glyphosate ha⁻¹). Abundance of *G. rubens* was not related to amount of dead vegetation, but was positively related to vegetation height (14%). There have been very few studies of *G. rubens*, however, it is found in the litter layer of grassland, heath and woodlands (McFerran *et al.*, 1994; Crocker & Daws, 1996) and appears to prefer undisturbed, well vegetated sites with a moderate soil moisture (Rushton *et al.*, 1987). The preference for these conditions may explain the reductions of the species under the relatively low rates of glyphosate application that caused decreasing vegetation height. Since humidity reduces as vegetation height decreases (de Keer *et al.*, 1989), the microclimate probably became increasingly unsuitable for this species. Mechanisms such as the avoidance of adverse conditions, including food deprivation (as detailed above for *L. tenuis*), may also play a part in the response of *G. rubens* to applications of broad-spectrum herbicides, such as glyphosate. *G. rubens* was found to be an indicator species of upland grassland habitats (Rushton & Eyre, 1992) and may be a useful indicator of herbicide treated field margins, due to its greater sensitivity to lower rates, however, the mechanisms for its response need clarifying.

7.4.2.3 Carabidae

The Carabidae were the least abundant of the three groups of arthropod studied in this experiment. The carabid beetles were not significantly affected by glyphosate applied at rates of equal to and less than 360g ha⁻¹, however, at rates of 360g glyphosate ha⁻¹ and

more, the abundance of Carabidae was significantly reduced in each of the glyphosate-treated plots compared with in the unsprayed plots. There is some discrepancy in these results, because in 1997 abundance of Carabidae in unsprayed plots and in plots treated with 360g glyphosate ha⁻¹ were not significantly different from each other, while in 1998, abundance was reduced by the 360g glyphosate ha⁻¹ treatment. Abundance of carabid beetles is thought to be governed by soil conditions rather than by vegetation (Luff & Rushton, 1988; Sanderson *et al.*, 1995) and since the experimental field margins in 1997 and 1998 were in different fields, differences in soil conditions may explain this inter-year discrepancy.

Although abundance of Carabidae was positively related to vegetation height and negatively related to amount of dead vegetation, these relationships were weak (explaining 2% and 5% of the variation respectively) and only present when the higher rates of glyphosate were applied, in 1998. This confirms that vegetation is not an important determinant of carabid beetle abundance (Sanderson *et al.*, 1995) and that other factors determined carabid abundance in the herbicide treated field margins.

Despite the clear reduction in total abundance of Carabidae in the experimental field margin plots treated with the higher rates of glyphosate in 1998, it was only possible to investigate the changes in abundance of two species due to the low numbers caught by this sampling method (as described above). Nevertheless, reductions in carabid abundance were recorded from these experimental plots and many studies have identified different phenologies, habitat preferences and feeding, overwintering and reproductive strategies in agro-ecosystem Carabidae that make it difficult to interpret causes for changes in abundance due to herbicide applications. For example, the distribution of *Demetrias atricapillus*, a predatory plant-climbing species (Forsythe, 1987), was weakly related to

both vegetation height (positively) and amount of dead vegetation (negatively) when glyphosate was applied at rates greater than 360g ha⁻¹, but abundance was not significantly different between treatments.

Differences in phenologies and reproductive behaviour may have contributed to differences in abundance of Carabidae. *Pterostichus melanarius* is an autumn breeder and uses field margin habitat as overwintering sites (Desender *et al.*, 1989) and may have migrated to unsprayed field margin habitat during the latter part of the sampling season.

The habitat preferences of species are also important in determining the impact of herbicide to field margins on Carabidae, since the ground beetles species recorded in this experiment represented field, hedgerow and associated-with-hedgerow species (Pollard, 1968a). For example, *Trechus quadristriatus*, which did not vary in abundance between treatments, was one of the many field species (Pollard, 1968a) recorded in this experiment. Thus, reductions in abundance of this species were not expected, since it not characteristic of a grassy field margin habitat.

Changes in the field margin vegetation caused by glyphosate applications were probably more wide ranging than those variables measured (height and dead plant material) and it is possible that other factors, such as stand density, were important in determining the carabid fauna of the glyphosate treated plots. Many species recorded from the experimental field margins were associated with tussock-grasses (e.g. *Dromius melanocephalus*, *D. linearis*, *Trechus obtusus*) and changes in their abundance due to changes in the quality of the tussock grasses in the field margins (e.g. *Dactylis glomerata*) would have been expected. However, some species are known to prefer dead stems of tussock grasses at some stage of their reproductive life and may have preferred tussocks desiccated by the herbicide. For

example, *D. linearis* was recorded in old stands of *D. glomerata*, where it lays eggs in the dead panicle stems (Luff, 1966).

Available food source is an important factor in distribution of arthropods, especially for egg and sperm production (McNeill & Southwood, 1978). Chiverton & Sotherton (1991) found that females of the autumn breeding *Pterostichus melanarius* from herbicide-sprayed cereal headlands were less satiated and less fecund than females from unsprayed areas. These reductions in reproductive fitness were shown to be related to decreases in available prey items and, although abundance of *P. melanarius* was not significantly different between the sprayed and unsprayed headlands (Chiverton & Sotherton, 1991), there are implications for future populations of this species in herbicide-treated habitat.

Differences in feeding strategy may explain changes in total abundance of Carabidae and it would be worth investigating this aspect. The two species that occurred in sufficiently high numbers for further analysis were both predators and were not affected by the high application rates of glyphosate. However, it is possible that many predatory species were affected by glyphosate applications, since Chiverton & Sotherton (1991) noted that prey items for species of Carabidae recorded in this experiment were dominated by linyphiid spiders, aphids, Heteroptera and adult Coleoptera. In this experiment, linyphiid spiders and the Heteroptera were significantly reduced in abundance under the higher rates of glyphosate, therefore, it is likely that prey availability for many predatory carabids was restricted. It may also be possible that the herbivorous species influenced the reduction in total abundance of Carabidae. Powell *et al.* (1985) found that herbicide applications to winter wheat reduced the abundance of the herbivorous *Amara* spp.. The herbivores, *Amara* and *Harpalus* spp. were recorded in this study and may have been affected by changes in food quality and availability caused by the glyphosate. It is suggested that

changes in prey availability for the predatory species may have contributed to the reduced abundance of carabid beetles in the plots treated with high levels of glyphosate.

The reductions in carabid abundance *per se* recorded in this experiment are supported by similar experiments in winter wheat (Brust, 1990) and a hedgerow (Pollard, 1968a), but causal mechanisms are yet to be identified. Asteraki *et al.* (1992) also investigated the effect of herbicide applications to hedgerow flora on Carabidae and through ordination techniques, suggested that herbicide altered the community structure, however, replication was low in their experiment and firm conclusions cannot be drawn. Thus, it is suggested that due to diverse feeding strategies, life histories and habitat requirements of this group, analysis of feeding guilds and autecologies be studied.

7.4.2.4 Heteroptera

Rates of glyphosate equal to and less than 360g ha⁻¹ had no significant effect on the abundance of all Heteroptera or on predatory Heteroptera, but did significantly reduce the abundance of phytophagous bugs in June and July. In June, there were significantly fewer phytophagous Heteroptera in the 180g and 360g treatments than in the 90g treatment, and in July there were significantly fewer phytophagous Heteroptera in each of the glyphosate treated plots than in the unsprayed plots. By August, there were no differences in abundance of phytophagous bugs between treatments. When rates of glyphosate were increased to more than 360g glyphosate ha⁻¹ in the second year, there were significantly fewer Heteroptera *per se* and phytophagous Heteroptera in the all glyphosate-treated plots than in the unsprayed plots, but abundance of predatory bugs did not differ significantly between the treatments.

The impact of glyphosate on total abundance of Heteroptera became more apparent when the rates of glyphosate were increased to above 360g ha⁻¹. Since this group contains examples of phytophages, predators and omnivores, reasons for its response to glyphosate may be complex. The abundance of predators and phytophages was more or less equal in this experiment, and it is likely that the increasing impact of higher rates of glyphosate on the phytophagous individuals (i.e. reducing abundance) became more influential on the total abundance of Heteroptera in the treatments, thereby reducing the overall abundance of Heteroptera. Furthermore, the omnivorous species may have found that food plants of a decreasing quality and quantity in the experimental field margins treated with the highest rates of glyphosate (more than 360g ha⁻¹) were no longer sufficient to supplement their varied diet. Studies of the effect of herbicide applications to crop headlands have also shown that abundance of heteropteran bugs is reduced (Chiverton & Sotherton, 1991; Sotherton & Moreby, 1992; Chiverton, 1999; Moreby & Southway, 1999) due to a decrease in weediness within the crop. Weeds within the crop not only provide an available food source for the phytophagous species, but also an ameliorated, more stable microclimate.

Changes in the micro-habitat and climatic conditions (e.g. reduced structural complexity and decreased humidity) caused by the applications of the herbicide are likely to have contributed to reduced abundance of all Heteroptera in the plots treated with more than 360g glyphosate ha⁻¹, since abundance was negatively related to vegetation height. Short vegetation is associated with reduced humidity and increased temperatures (de Keer *et al.*, 1989) and vegetation height, together with its associated structural complexity and microclimate are important factors in determining Heteroptera abundance and diversity (Gibson *et al.*, 1992b; Fauvel, 1999). Indeed, changes in Heteroptera faunal abundance and diversity associated with changes in vegetation height have been recorded from

grasslands that had been subjected to cutting (Morris & Lakhani, 1979) and grazing (Morris, 1973) regimes.

It appeared from this experiment that the effects of glyphosate applied at lower rates on phytophagous heteropterans were relatively ephemeral, while effects of the higher rates are more prolonged. These results for the phytophagous heteropterans correspond well with the effects of lower rates of glyphosate on the vegetation height and health, where increased rates of glyphosate were associated with shorter, less healthy vegetation. Furthermore, abundance of phytophagous Heteroptera was positively related to vegetation height and negatively related to amount of dead vegetation, where amount of dead vegetation was more significant in determining abundance (Table 7.20), indicating that food plant quality and availability had been compromised. Similar effects of herbicide applications to cereal headlands were found by Moreby (1994) where grass feeding bugs (*Stenodemini*) were reduced by herbicide applications, although not significantly.

These effects of glyphosate on the abundance of the phytophagous Heteroptera were not unexpected, since they rely on an abundant and rich food source in addition to appropriate microclimatic conditions (Dolling, 1991). The composition of Heteroptera fauna is known to depend on several factors, including microclimate and the existence of vegetation with several strata (Fauvel, 1999). Taller vegetation, for example provides complex and varied strata and a more humid microclimate, which serves to maintain hydration in Heteroptera (McNeill, 1973). Vegetation that has not been exposed to, or is not susceptible to plant protection products provides a diversity of food plants and therefore nutrition, necessary for development and fecundity (Southwood & Leston, 1959; Fauvel, 1999).

The impact of glyphosate on the predatory Heteroptera, however, seems to be negligible, although Pollard (1968b) recorded a significant decrease in the abundance of predatory Heteroptera in a hedgerow that had had the ground flora chemically removed. The results obtained in this study may be a reflection of the small plot sizes (12m long), thus if more resources had been available, larger experimental plots would have more appropriate. Nevertheless, there were no significant differences in abundance of predatory heteropteran bugs between the treatments and although abundance was positively related to vegetation height and negatively related to plant health, the strength of the relationships were very low (all less than 15%). What is not apparent, however, is the impact of the changes in habitat on feeding behaviour and subsequent development, mating success and fecundity of the predatory bugs. It is known, for example, that daily fecundity in Heteroptera varies according to many factors, including in particular, food quantity and quality (Fauvel, 1999). If prey items were reduced (abundance of many arthropod groups in this experiment were reduced) by increasing rates of glyphosate, this may have implications for future populations of these predators.

7.4.2.5 Community Responses

The Araneae, Carabidae and Heteroptera community data were not analysed individually, since useful interpretation of the low abundance of the Heteroptera and low abundance and diversity of the Carabidae was not possible. However, the combined data set was more robust and provided a clearer indication of the effects of glyphosate applications to a wider arthropod community. The community analyses summarise the results for the combined groups of arthropod. The ordination plot (Figure 7.18) indicates that when glyphosate was applied at rates equal to and less than 360g ha⁻¹, the spider, carabid and Heteroptera community in the treated plots were not markedly different from each other, although the

community in the 360g treatment was slightly separated from the main cluster along axis 1. Axis 1 was negatively correlated with vegetation height, indicating that the communities in the field margin plots treated with 360g glyphosate ha⁻¹, which were at the upper of the scale of Axis 1, were associated with shorter vegetation. Although Axis 1 did not correlate with rate of glyphosate, increasing the rate of glyphosate was shown to reduce vegetation height, therefore, it is logical to assume that higher rates of glyphosate would result in a more different community structure than the field margin plots sprayed with lower rates of glyphosate or those that were left unsprayed.

When higher rates of glyphosate were applied to the field margin plots, there was stronger separation of communities from the treatments along axis 1 of the DCA plot (Figure 7.19). The communities of the glyphosate-treated field margin plots were clustered, indicating that they were similar. The communities from the unsprayed plots, however, were separated from the main cluster, indicating that they were different from the communities of the sprayed plots. Axis 1 was strongly positively correlated with amount of dead vegetation and rate of glyphosate and strongly negatively correlated with vegetation height. Thus, communities at the lower end of the scale of Axis 1 were associated with taller vegetation, minimal cover by dead plant material and lowest rates of glyphosate and here, these communities were those in the unsprayed control plots.

These results show that applications of glyphosate at rates of 360g ha⁻¹ and more can change the structure and composition of the Araneae, Heteroptera and Carabidae community and that these changes are significantly related to vegetation height and cover by dead plant material. DCA has been used by many authors to simplify arthropod community data so that trends in the species composition can be more easily described (e.g., Gibson *et al.*, 1992a; Rushton & Eyre, 1992; Sanderson *et al.*, 1995). Rushton *et al.*

(1987) also correlated measured environmental variables against axis scores, in order to determine the relationship between the variables and the grassland spider communities and found that management regime (intensity of grazing) and site wetness were major influential factors. Gibson *et al.* (1992a) also found that spider communities were influenced by grazing regime, where communities in heavily grazed areas, where swards were short and compact, were distinct from both more lightly grazed and ungrazed grassland plots.

There appears, from the analyses on the three individual groups, that various factors influence the reductions in abundance of Araneae, Carabidae and Heteroptera in field margins treated with glyphosate. The ordination plots confirm that where glyphosate is applied at more than 360g ha⁻¹, these reductions result in different assemblages to unsprayed field margin plots.

7.4.2.6 Longer Term Effects of Glyphosate

In order to obtain an indication of the longer term effects of glyphosate applications on Araneae, Carabidae and Heteroptera, arthropod and vegetation sampling was repeated in the unsprayed control and 360g glyphosate ha⁻¹ experimental plots at 12 and 16 months after herbicide application (May and September 1998). The abundance of spiders, Carabidae, Heteroptera, phytophagous and predatory Heteroptera did not significantly differ between the two treatments at either 12 or 16 months after spray application and there was no association between rate of glyphosate and vegetation height or dead plant material.

There was only one species of arthropod affected by the glyphosate one year after treatment: abundance of *Lepthyphantes ericaeus* was significantly reduced in the 360g glyphosate ha⁻¹ treatment compared with the unsprayed control at 12 months after the spray application only. *L. ericaeus* is a linyphiid spider, which builds small horizontal sheet webs near ground level (Duffey 1966) in long, often damp grassland where there is an abundance of litter (Duffey, 1962; 1963). Since there were no associations between rate of glyphosate and vegetation height or cover by dead plant material, other factors, such as density of vegetation, must have influenced the differences in abundance of *L. ericaeus*. However, this species has an affinity with damp grassland (Crocker & Daws, 1996) and may be particularly sensitive to reduced humidity levels caused by previous applications of glyphosate. Although the causes of the reduced abundance of *L. ericaeus* in the 360g glyphosate ha⁻¹ field margin plots 12 months after spray application are not clear, this illustrates that herbicide effects can be long term.

The initial impact of herbicides on non-target arthropods is exposure to the active ingredient, which can occur through direct contact, or through indirect contact with the active ingredient by, for example, consumption of contaminated food source. The patterns of spray drift intercepted by commonly occurring field margin plant species in fully sprayed strips, and protected by 2m and 6m wide buffer strips were measured (Chapter 3) to give i) an indication of the amount of herbicide likely to be encountered by arthropods using the vegetation and, ii) precise amounts of active ingredient available for uptake by plant species. The fully sprayed strip simulated boundary vegetation adjacent to fully sprayed crops, while the 2m and 6m wide buffer strips simulated conservation field margins offered under the Countryside Stewardship Scheme (MAFF, 1996) and the pilot Arable Stewardship (MAFF, 1998) Schemes respectively. It was found that exposure to spray drift was exponentially reduced with increasing distance from the sprayer, where an average of 15% and 5% of spray intercepted in the fully sprayed strip was intercepted by plants protected by the 2m and 6m wide buffer strips respectively.

However, there were interspecific differences in the amounts of spray drift intercepted, where some species (*Arrhenatherum elatius*, *Rumex obtusifolius* and *Festuca rubra*) intercepted more drift than the other test species and taller plants intercepted more drift than shorter plants when protected by the 2m and 6m buffer strips. Furthermore, the 2m and 6m wide buffer strips did not significantly reduce the interception of drift by *Dactylis glomerata* and *Cirsium vulgare* respectively. Therefore, these species could be at more risk from effects of herbicide drift than others, and this depended upon the inherent susceptibility of the species to glyphosate (Chapter 4), which will be discussed below.

To determine the effects on arthropods of direct and residual exposure to glyphosate under fully sprayed conditions, toxicity of testing of different rates was carried out (Chapter 6). It was determined that glyphosate was non-toxic to *Lepthyphantes tenuis* (Araneae: Linyphiidae) and apparently non-toxic to adult and juvenile *Leptopterna dolabrata* (Heteroptera: Miridae), although experimental conditions for *L. dolabrata* required refining before conclusive non-toxic status of glyphosate can be assured for this species. Because glyphosate had no insecticidal properties, it was concluded that any effects of glyphosate on abundance of arthropods must be indirect.

Indirect exposure of arthropods to glyphosate (e.g. contaminated food source) was not investigated, since there were no apparent effects of direct exposure. Indirect effects, however, were examined in detail. The effect of glyphosate applications to food plants on maturing *L. dolabrata* were investigated, in order to determine whether the action of the herbicide altered the quality of the food source (Chapter 5). This experiment inferred that there may be significant disruptions to the quality of food plant, since mortality of *L. dolabrata* feeding from glyphosate-treated plants was greater than that of the species feeding from unsprayed plants.

Indirect effects of herbicide on arthropods are a function of the direct effects on vegetation, therefore, the inherent susceptibility of commonly occurring field margin plant species was studied in dose-response testing (Chapter 4). Even under the highest rate of glyphosate, two species neither showed strong signs of damage nor suffered from reduced growth (*Geranium robertianum* and *Festuca rubra*). Other species were visually damaged (*Centaurea nigra*, *Elymus repens* and *Silene alba*), but their growth was not affected. The implications of damaged, but growing plants for arthropods relate to phytophagous species, since although plants may continue to grow, visible damage may be a reflection of areas of

the plant surface that are unsuitable for feeding.

It was possible to predict ED₅₀ doses for many of the plant species that did respond to the glyphosate. These data were combined with those from the spray drift experiment to predict percentage spray volume of applications to the crop that could reach the species in the field boundary (0m) and adjacent to the 2m and 6m wide buffer strips (Table 8.1). These values were used to estimate possible effects of drift onto plants in the field boundary (0m) and protected by 2m and 6m wide buffer strips of the lowest commonly used, typical and maximum UK recommended rates of glyphosate (360g, 1080g and 2160g ha⁻¹, Anon, 1999b) applied in British farmland (Table 8.2).

Table 8.1. Predicted percentage volume of field-applied spray that could be intercepted by plant species in the field boundary (0m) and adjacent to 2m and 6m wide buffer strips.

	Percent (x 10 ⁻³) of field rate reaching species		
	0m	2m	6m
<i>Agrostis stolonifera</i>	49.6	4.3	1.6
<i>Cerastium holosteoides</i>	33.9	1.3	0.6
<i>Cirsium arvense</i>	65.6	5.1	1.7
<i>Cirsium vulgare</i>	140.4	10.7	4.5
<i>Dactylis glomerata</i>	164.8	40.0	9.4
<i>Tripleurospermum maritimum</i>	74.3	9.4	5.1

Table 8.2. Predicted amount of glyphosate reaching plant species in the field boundary and adjacent to 2m and 6m wide buffer strips when applied at recommended rates in the crop compared with ED₅₀ values. Note predicted amounts of glyphosate are x 10⁻³.

	Predicted Amount of Glyphosate Reaching Plant Species (g ha ⁻¹ x 10 ⁻³)									ED ₅₀ (g ha ⁻¹)
	360g			1080g			2160g			
	0m	2m	6m	0m	2m	6m	0m	2m	6m	
<i>A. stolonifera</i>	178.6	15.5	5.8	535.7	46.4	17.3	1071.4	92.88	34.56	751.7
<i>C. holosteoides</i>	122.0	4.7	2.2	366.1	14.0	6.5	732.2	28.08	13.0	527.4
<i>C. arvense</i>	236.2	18.4	6.1	708.5	55.1	18.4	1417.0	110.16	36.7	925.2
<i>C. vulgare</i>	505.4	38.5	16.2	1516.3	115.6	48.6	3032.6	231.12	97.2	1453.0
<i>D. glomerata</i>	593.3	144.0	33.8	1779.8	432.0	101.5	3559.7	864.00	203.0	2098.1
<i>T. maritimum</i>	267.5	33.8	18.4	802.4	101.5	55.1	1604.9	203.04	110.2	1097.6

It appears that none of the species would suffer significant damage from glyphosate drift at these doses, because the predicted amounts of glyphosate reaching the plant species in the field boundary and protected by the 2m and 6m wide buffer strips are well below the ED₅₀s. However, the wind speed during the spray drift experiment was low and drift will have been minimal: in practice herbicide applications are often carried-out under higher wind speeds and affects of herbicide spray drift on non-target vegetation are frequently seen (e.g. Figure 1.1). Furthermore, it was noted that the results from the dose-response experiment (Chapter 4) may not be a true reflection of effects of high rates of glyphosate, since the plants were not subjected to inter- and intraspecific competition and may not have been fully actively growing at spray application.

The indirect effects of glyphosate on non-target field margin arthropods were studied in a field experiment (Chapter 7) to quantify changes in abundance of groups and species of arthropod. Different rates of glyphosate were applied to experimental grassy field margins that were believed to represent rates of drift and also direct application rates of glyphosate in the UK. Glyphosate applied at rates greater than 90g ha⁻¹ reduced the abundance of phytophagous Heteroptera, while rates greater than 180g ha⁻¹ reduced the abundance of

Gonatium rubens (Araneae: Linyphiidae). Rates of more than 360g ha⁻¹ had the greatest implications for the arthropod groups studied: abundance of total Araneae, Carabidae, Heteroptera and *Lepthyphantes tenuis* (Araneae: Linyphiidae) was significantly reduced, and the community structure of the Araneae, Carabidae and Heteroptera was different to that from the unsprayed field margins. Perhaps even more significant was the delayed and prolonged effect of glyphosate applied at 360g ha⁻¹ on *Lepthyphantes ericaeus* 12 months after application, where abundance was significantly reduced compared with in the untreated plots.

The changes in abundance of arthropods were related to changes in vegetation characteristics and associated conditions. Vegetation height and cover by unpalatable dead vegetation were important in determining distribution of Araneae, *L. tenuis*, *G. rubens*, Heteroptera and phytophagous Heteroptera. It is suggested that changes in the abundance and availability of prey items and modifications of the microclimate and structural features were important for the Carabidae and Araneae.

Predictions of effects of glyphosate drift into field margins on non-target arthropods can be extrapolated from the experimental data, based on reductions in spray drift intercepted by all plants in the field boundary (0m) and adjacent to 2m and 6m wide buffer strips (Chapter 3; Table 8.3). The full rates are based on those recommended for use for weed control in the UK, however, the most commonly used rates are greater than 360g ha⁻¹ (Anon, 1999b). These predictions suggest that small amounts of glyphosate would reach non-target vegetation under the calm spraying conditions experienced in the spray drift experiment (Chapter 3). Predicted amounts of glyphosate drift reaching vegetation *per se* are greater than for individual species (Table 8.2), since mean drift interception by all plants was greater (Chapter 3). These predicted levels of glyphosate drift infer that while arthropods

protected by the 2m and 6m wide buffer strips would not be significantly affected, some groups in the field boundary would be reduced in abundance. The most vulnerable groups would be the phytophagous Heteroptera and *G. rubens*, since they were significantly reduced in abundance by rates of more than 90g glyphosate ha⁻¹ and 180g ha⁻¹ respectively (Chapter 7). More significantly, glyphosate applied at 1080g and 1440g ha⁻¹ are amongst the most commonly used rates in UK arable ecosystems and their longer term impacts on field margin arthropod biodiversity and higher trophic level taxa may be significant.

Table 8.3. Predicted rate of glyphosate reaching vegetation in field boundaries (0m) and adjacent to 2m and 6m wide buffer strips when applied to the crop at UK recommended field rates.

Full Rate of Glyphosate (g ha ⁻¹) Applied to Crop	Predicted Rate of Glyphosate drift (g ha ⁻¹)		
	0m	2m	6m
90	13.76	2.06	0.69
180	27.52	4.13	1.38
360	55.05	8.26	2.75
720	110.09	16.51	5.50
1080	165.14	24.77	8.26
1440	220.18	33.03	11.01
2160	330.28	49.54	16.51

9 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDY

This series of experiments illustrated that although glyphosate is apparently non-toxic, it changes the quality of food plants. Furthermore, levels of herbicide drift can be significantly reduced by inclusions of 2m and 6m wide buffer strips. It is important to note, though, that these buffer strips do not provide such high levels of protection to those species growing in the buffer areas, although an inclusion of a narrow strip of tall vegetation, especially *D. glomerata* might be useful in intercepting drift, thereby preventing it from reaching non-target habitat. Although some species appeared to be unaffected by even high levels of glyphosate and others had relatively high ED₅₀s, many of the plants are important as food sources and structural components within field margins, and may, under refined experimental conditions be found to be more susceptible to glyphosate than suggested here. The arthropod fauna is reduced in abundance and community structure is altered when rates of glyphosate more than 360g ha⁻¹ come into contact with grassy field margins, where Araneae (mainly Linyphiidae), Carabidae, and phytophagous Heteroptera are particularly at risk.

What is still not clear, however, is what the indirect effects of glyphosate on vegetation are to arthropods. For example, abundance, quality and availability of prey items for predatory species need to be assessed, as do the impacts of changes in vegetation architecture and microclimate on arthropods *per se*. Determining accurate ED₅₀s for the plant species tested here needs to be re-assessed to predict effects of glyphosate drift. Also refining experiment conditions for *L. dolabrata* would enable one to identify any insecticidal properties of glyphosate and also effects on food plant quality. Furthermore, the ways in which glyphosate-treated food plants are altered need to be quantified, since the experiment here suggested that food plant quality may be impaired.

10 REFERENCES

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**APPENDIX 1: LIST OF PLANT SPECIES RECORDED FROM
THE FIELD MARGINS AT LODDINGTON IN THIS STUDY**

Taxonomic List

Juncaceae

Juncus inflexus L.

Gramineae

Bromus sterilis L.

Bromus mollis L.

Elymus repens (L.)

Festuca rubra L.

Dactylis glomerata L.

Arrhenatherum elatius (L.)

Holcus lanatus L.

Agrostis stolonifera L.

Alopecurus pratensis L.

Cruciferae

Alliaria petiolata (Bieb.)

Caryophyllaceae

Cerastium fontanum Baumg.

Geraniaceae

Geranium robertianum L.

Geranium molle L.

Rosaceae

Prunus spinosa L.

Crataegus laevigata (Poiret)

Rosa canina agg L.

Rubus fruticosus agg L.

Onagraceae

Epilobium spp L.

Urticaceae

Urtica dioica L.

Araliaceae

Hedera helix L.

Aceraceae

Acer campestre L.

Umbelliferae

Angelica sylvestris L.

Heracleum sphondylium L.

Polygonaceae

Rumex obtusifolius AUTH

Scrophulariaceae

Veronica persica Poiret

Labiatae

Lamium purpureum L.

Lamium album L.

Glechoma hederacea L.

Stachys sylvatica L.

Stachys arvensis (L.)

Boraginaceae

Myosotis arvensis (L.)

Caprifoliaceae

Sambucus nigra L.

Rubiaceae

Galium aparine L.

Compositae

Senecio jacobaea L.

Achillea millefolium L.

Cirsium vulgare (Savi)

Cirsium arvense (L.)

Alphabetical List

Acer campestre L.

Achillea millefolium L.

Agrostis stolonifera L.

Alliaria petiolata (Bieb.)

Alopecurus pratensis L.
Angelica sylvestris L.
Arrhenatherum elatius (L.)

Bromus mollis L.
Bromus sterilis L.

Cerastium fontanum Baumg.
Cirsium arvense (L.)
Cirsium vulgare (Savi)
Crataegus laevigata (Poiret)

Dactylis glomerata L.

Elymus repens (L.)
Epilobium spp L.

Festuca rubra L.

Galium aparine L.
Geranium molle L.
Geranium robertianum L.
Glechoma hederacea L.

Hedera helix L.
Heracleum sphondylium L.
Holcus lanatus L.

Juncus inflexus L.

Lamium album L.
Lamium purpureum L.

Myosotis arvensis (L.)

Prunus spinosa L.

Rosa canina agg L.
Rubus fruticosus agg L.

Sambucus nigra L.
Senecio jacobaea L.
Stachys arvensis (L.)
Stachys sylvatica L.

Urtica dioica L.
Veronica persica Poiret

APPENDIX 2: ARANEAE SPECIES RECORDED FROM THE FIELD MARGINS AT LODDINGTON IN THIS STUDY

Taxonomic List

Oonopidae

Oonops domesticus (de Dalmas)

Gnaphosidae

Micaria pulicaria (Sundevall)

Clubionidae

Clubiona reclusa Cambridge

Clubiona lutescens Westring

Clubiona compta Koch

Zoridae

Zora spinimana (Sundevall)

Thomisidae

Xysticus cristatus (Clerck)

Ozyptila praticola (Koch)

Philodromidae

Philodromus dispar Walckenaer

Philodromus cespitum Walckenaer

Philodromus collinus Koch

Tibellus oblongus Walckenaer

Salticidae

Euophrys frontalis Walckenaer

Lycosidae

Pardosa palustris (L.)

Pardosa pullata (Clerck)

Pardosa prativaga (Koch)

Pardosa amentata (Clerck)

Pardosa nigriceps (Thorell)

Alopecosa pulverulenta (Clerck)

Trochosa ruricola (Degeer)

Trochosa terricola Thorell

Pisauridae

Pisaura mirabilis (Clerck)

Mimetidae

Ero cambridgei Kulczynski

Ero furcata (Villers)

Theridiidae

Episinus angulatus (Blackwall)

Theridion bimaculatum (L.)

Enoplognatha ovata (Clerck)

Robertus lividus (Blackwall)

Pholcomma gibbum (Westring)

Tetragnathidae

Tetragnatha extensa (L.)

Tetragnatha montana Simon

Pachygnatha clercki Sundevall

Pachygnatha degeeri Sundevall

Meta segmentata (Clerck)

Meta mengei (Blackwall)

Araneidae

Larinioides cornutus (Clerck)

Araniella opistographa (Kulczynski)

Linyphiidae

Ceratinella brevipes (Westring)

Ceratinella scabrosa (Cambridge)

Walckenaeria acuminata Blackwall

Walckenaeria nudipalpis (Westring)

Walckenaeria unicornis Cambridge

Walckenaeria cuspidata (Blackwall)

Dicymbium nigrum (Blackwall)

Entelecara erythropus (Westring)

Gongylidiellum vivum (Sundevall)

Dismodicus bifrons (Blackwall)

Gonatium rubens (Blackwall)

Maso sundevalli (Westring)

Pocadicnemis juncea Locket & Millidge

Oedothorax fuscus (Blackwall)

Oedothorax retusus (Westring)

Cnephalocotes obscurus (Blackwall)

Linyphiidae (cont)

Monocephalus fuscipes (Blackwall)
Micrargus herbigradus (Blackwall)
Micargus subaequalis (Westring)
Erigonella hiemalis (Blackwall)
Savignya frontata (Blackwall)
Diplocephalus latifrons (Cambridge)
Diplocephalus connatus Bertkau
Araeoncus humilis (Blackwall)
Panamomops sulcifrons (Wider)
Erigone dentipalpis (Wider)
Erigone atra (Blackwall)
Porrhomma microphthalmum
(Cambridge)
Meioneta rurestris (Koch)
Meioneta saxatilis (Blackwall)
Syedra gracilis (Menge)
Centromerus sylvaticus (Blackwall)
Centromerita bicolor (Blackwall)
Bathyphantes gracilis (Blackwall)
Bathyphantes parvulus (Westring)
Diplostyla concolor (Wider)
Poeciloneta globosa (Wider)
Stemonyphantes lineatus (L.)
Lepthyphantes tenuis (Blackwall)
Lepthyphantes mengei Kulczynski
Lepthyphantes ericaeus (Blackwall)
Lepthyphantes pallidus (Cambridge)
Lepthyphantes insignis Cambridge
Neriere clathrata (Sundevall)
Microlinyphia pusilla (Sundevall)

Alphabetical List

Alopecosa pulverulenta (Clerck)
Araeoncus humilis (Blackwall)
Araniella opistographa (Kulczynski)

Bathyphantes gracilis (Blackwall)
Bathyphantes parvulus (Westring)

Centromerita bicolor (Blackwall)
Centromerus sylvaticus (Blackwall)
Ceratinella brevipes (Westring)
Ceratinella scabrosa (Cambridge)
Clubiona compta Koch

Clubiona lutescens Westring
Clubiona reclusa Cambridge
Cnephalocotes obscurus (Blackwall)

Dicymbium nigrum (Blackwall)
Diplocephalus latifrons (Cambridge)
Diplocephalus connatus Bertkau
Diplostyla concolor (Wider)
Dismodicus bifrons (Blackwall)

Enoplognatha ovata (Clerck)
Entelecara erythropus (Westring)
Episinus angulatus (Blackwall)
Erigone atra (Blackwall)
Erigone dentipalpis (Wider)
Erigonella hiemalis (Blackwall)
Ero cambridgei Kulczynski
Ero furcata (Villers)
Euophrys frontalis Walckenaer

Gonatium rubens (Blackwall)
Gongylidiellum vivum (Sundevall)

Larinioides cornutus (Clerck)
Lepthyphantes ericaeus (Blackwall)
Lepthyphantes insignis Cambridge
Lepthyphantes mengei Kulczynski
Lepthyphantes pallidus (Cambridge)
Lepthyphantes tenuis (Blackwall)

Maso sundevalli (Westring)
Meioneta rurestris (Koch)
Meioneta saxatilis (Blackwall)
Meta mengei (Blackwall)
Meta segmentata (Clerck)
Micaria pulicaria (Sundevall)
Micrargus herbigradus (Blackwall)
Micargus subaequalis (Westring)
Microlinyphia pusilla (Sundevall)
Monocephalus fuscipes (Blackwall)
Neriere clathrata (Sundevall)

Oedothorax fuscus (Blackwall)
Oedothorax retusus (Westring)
Oonops domesticus (de Dalmas)

Ozyptila praticola (Koch)

Pachygnatha clercki Sundevall

Pachygnatha degeeri Sundevall

Panamomops sulcifrons (Wider)

Pardosa amentata (Clerck)

Pardosa nigriceps (Thorell)

Pardosa palustris (L.)

Pardosa prativaga (Koch)

Pardosa pullata (Clerck)

Philodromus dispar Walckenaer

Philodromus cespitum Walckenaer

Philodromus collinus Koch

Pholcomma gibbum (Westring)

Pisaura mirabilis (Clerck)

Pocadicnemis juncea Locket & Millidge

Poeciloneta globosa (Wider)

Porrhomma microphthalmum

(Cambridge)

Robertus lividus (Blackwall)

Savignya frontata (Blackwall)

Stemonyphantes lineatus (L.)

Syedra gracilis (Menge)

Tetragnatha extensa (L.)

Tetragnatha montana Simon

Theridion bimaculatum (L.)

Tibellus oblongus Walckenaer

Trochosa ruricola (Degeer)

Trochosa terricola Thorell

Walckenaeria acuminata Blackwall

Walckenaeria cuspidata(Blackwall)

Walckenaeria nudipalpis (Westring)

Walckenaeria unicornis Cambridge

Xysticus cristatus (Clerck)

Zora spinimana (Sundevall)

APPENDIX 3: CARABIDAE SPECIES RECORDED FROM
THE FIELD MARGINS AT LODDINGTON IN THIS STUDY

Taxonomic List

Leistus ferrugineus L.
Notiophilus biguttatus Fab.
Notiophilus palustris Duftschmid
Trechus secalis Paykull
Trechus quadristriatus Schrank
Trechus obtusus Erichson
Bembidion lampros
Bembidion guttula
Pterostichus cupreus L.
Pterostichus melanarius Illiger
Pterostichus nigrita Paykull
Pterostichus strenuus Panzer
Agonum dorsale Ponoppidan
Amara plebeja Gyllenhal
Amara bifrons Gyllenhal
Amara familiaris Duftschmid
Harpalus rufipes DeGeer
Harpalus rufibarbis Fab.
Trichocellus placidus Gyllenhal
Bradycellus verbasci Duftschmid
Badister bipustulatus Fab.
Demetrias atricapillus L.
Dromius linearis Olivier
Dromius melanocephalus Dejean

Alphabetical List

Agonum dorsale Ponoppidan
Amara bifrons Gyllenhal
Amara familiaris Duftschmid
Amara plebeja Gyllenhal

Badister bipustulatus Fab.
Bembidion guttula
Bembidion lampros
Bradycellus verbasci Duftschmid

Demetrias atricapillus L.
Dromius linearis Olivier
Dromius melanocephalus Dejean

Harpalus rufibarbis Fab.
Harpalus rufipes DeGeer

Leistus ferrugineus L.

Notiophilus biguttatus Fab.
Notiophilus palustris Duftschmid

Pterostichus cupreus L.
Pterostichus melanarius Illiger
Pterostichus nigrita Paykull
Pterostichus strenuus Panzer

Trechus obtusus Erichson
Trechus quadristriatus Schrank
Trechus secalis Paykull
Trichocellus placidus Gyllenhal

APPENDIX 4: HETEROPTERA SPECIES RECORDED FROM THE FIELD MARGINS AT LODDINGTON IN THIS STUDY

Taxonomic List

Pentatomidae

Eysarcoris fabricii (Kirkaldy)

Rhopalidae

Rhopalus subrufus (Gmelin)

Lygaeidae

Heterogaster urticae (Fab.)

Ischnodemus sabuleti (Fallén)

Peritrechus geniculatus (Hahn)

Stygnocoris fuliginosus (Geoffroy)

Stygnocoris sabulosus (Fallén)

Drymus sylvaticus (Fab.)

Scolopostethus affinis (Schilling)

Scolopostethus decoratus (Hahn)

Scolopostethus thomsoni Reuter

Taphropeltus contractus (Herrich-Schäffer)

Cymus melanocephalus Fieber

Berytinidae

Berytinus clavipes (Fab.)

Berytinus minor (Herrich-Schäffer)

Piesma maculatum (Laporte)

Tingidae

Tingis ampliata (Herrich-Schäffer)

Tingis cardui (L.)

Nabidae

Nabis fesus (L.)

Nabis rugosus (L.)

Anaptus major (Costa)

Nabigula limbata (Dahlbom)

Anthocoridae

Anthocoris nemorum (L.)

Xylocoris galactinus (Fallén)

Miridae

Deraeocoris ruber (L.)

Amblytylus nastutus (Kirschbaum)

Orthonotus rufifrons (Fallén)

Plagiognathus arbustorum (Fab.)

Plagiognathus chrysanthemi (Wolff)

Dichyphus epilobii Reuter

Dichyphus stachydis Reuter

Heterotoma merioptera (Scopoli)

Pithanus maerkeli (Herrich-Schäffer)

Lygus rugulipennis Poppius

Lygocoris limbatus (Fallén)

Calocoris stysi (Fab.)

Calocoris norvegicus (Gmelin)

Phytocoris ulmi (L.)

Phytocoris varipes Boheman

Capsus ater (L.)

Stenodema calcaratum (Fallén)

Stenodema laevigatum (L.)

Notostira elongata (Geoffroy)

Megaloceraea recticornis (Geoffroy)

Leptopterna dolabrata (L.)

Alphabetical List

Amblytylus nastutus (Kirschbaum)

Anaptus major (Costa)

Anthocoris nemorum (L.)

Berytinus clavipes (Fab.)

Berytinus minor (Herrich-Schäffer)

Calocoris norvegicus (Gmelin)

Calocoris stysi (Fab.)

Capsus ater (L.)

Cymus melanocephalus Fieber

Deraeocoris ruber (L.)

Dichyphus epilobii Reuter

Dichyphus stachydis Reuter

Drymus sylvaticus (Fab.)

Eysarcoris fabricii (Kirkaldy)

Heterogaster urticae (Fab.)
Heterotoma merioptera (Scopoli)

Ischnodemus sabuleti (Fall9n)

Leptopterna dolabrata (L.)
Lygocoris limbatus (Fall9n)
Lygus rugulipennis Poppius

Megaloceraea recticornis (Geoffroy)

Nabacula limbata (Dahlbom)
Nabis fesus (L.)
Nabis rugosus (L.)
Notostira elongata (Geoffroy)

Orthonotus rufifrons (Fall9n)

Peritrechus geniculatus (Hahn)
Phytocoris ulmi (L.)
Phytocoris varipes Boheman
Piesma maculatum (Laporte)
Pithanus maerkeli (Herrich-Sch  ffer)
Plagiognathus arbustorum (Fab.)
Plagiognathus chrysanthemi (Wolff)

Rhopalus subrufus (Gmelin)

Scolopostethus affinis (Schilling)
Scolopostethus decoratus (Hahn)
Scolopostethus thomsoni Reuter
Stenodema calcaratum (Fall9n)
Stenodema laevigatum (L.)
Stygnocoris fuliginus (Geoffroy)
Stygnocoris sabulosus (Fall9n)

Taphropeltus contractus (Herrich-Sch  ffer)

Tingis ampliata (Herrich-Sch  ffer)
Tingis cardui (L.)

Xylocoris galactinus (Fall9n)